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ORIGINAL PAPER

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Interactions between photoautotrophic and heterotrophic metabolism in photoheterotrophic cultures of *Euglena gracilis*

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Abstract Interactions between photoautotrophic and heterotrophic metabolism in photoheterotrophic culture of *Euglena gracilis* were studied. Under a low light supply coefficient, these two metabolic activities seem to proceed independently. The cell growth rate in photoheterotrophic culture was about the sum of the growth rates in pure photoautotrophic and heterotrophic cultures. However under a high light supply coefficient, both photoautotrophic and heterotrophic (glucose assimilation) metabolic activities were inhibited, resulting in a low photoheterotrophic growth rate. The photoheterotrophic culture was more sensitive to photoinhibition compared to the pure photoautotrophic culture. Inhibition of glucose assimilation in the photoheterotrophic culture was due to both direct and indirect (through photosynthesis) effects of high light intensity. Cell growth, glucose assimilation and α-tocopherol content of the cells were higher when ambient air was used for aeration than when a mixture of carbon dioxide and air was used. Even when photosynthesis was inhibited by addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea to photoheterotrophic culture, light stimulated α-tocopherol synthesis by *E. gracilis*.

Introduction

The unicellular alga *Euglena gracilis* has great potential for the production of various metabolites. It is one of the few microorganisms which simultaneously produces antioxidant vitamins such as β-carotene, L-ascorbic acid and α-tocopherol (Takeyama et al. 1997). Depending on the culture condition, it can also accumulate significant amounts of wax esters (Inui et al. 1983); paramylum (Barras and Stone 1968) and highly unsaturated fatty acids (Hayashi et al. 1993). Furthermore, it has a very high nutritive value (Kitaoka and Hosotani 1977) and thus can be used as a dietary supplement in food and feeds. α-Tocopherol is a useful antioxidant in clinical and nutritional fields that may function in preventing various diseases caused by oxidative damage. α-Tocopherol production by *E. gracilis* was found to be the highest among the ~285 strains from more than 56 genera of microorganisms that were tested (Tani and Tsuruma 1989). *Euglena* can accumulate as much as 5 to 7 mg α-tocopherol (g cell)$^{-1}$ (Ruggeri et al. 1985; Tani and Osuka 1989; Tani and Tsuruma 1989). This compares with less than 0.3 mg g^{-1} found in vegetable oils and other plant materials. Furthermore, the more active α-tocopherol constitutes more than 97% of the total tocopherol in *Euglena*. Thus, α-tocopherol production by *Euglena* can substitute for the current chemically synthesized racemate of α-tocopherol or the mixture of α-, β(γ)- and δ-tocopherols extracted from vegetable oils.

Euglena can grow photoautotrophically, heterotrophically or photoheterotrophically. However, like other photosynthetic microorganisms, growth is limited by light under photoautotrophic conditions, while under heterotrophic culture, tocopherol synthesis is low. Consequently, two-step culture methods in which the cells are first cultivated photoheterotrophically (Takeyama et al. 1997) or heterotrophically (Ogbonna et al. 1999) and then subjected to photoautotrophic conditions for accumulation of the desired metabolites have been proposed. Also, a cyclic photoautotrophic-heterotrophic cultivation system, in which the cells are cultivated photoautotrophically during the day and heterotrophically during the night, has been proposed to overcome the problem of night biomass loss and to achieve continuous cell growth under light-dark cycles (Ogbonna and Tanaka 1998).

Despite the much higher productivities achieved with these cultivation systems, the production costs are still too high to compete with the current sources. One possible method of reducing the cost of tocopherol production by *E. gracilis* is to use wastewater as the culture medium. Many domestic and industrial wastewaters contain various carbon and nitrogen sources that can be metabolized by

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Euglena. For example, it has been reported that *Euglena* can metabolize many organic substrates such as acetate, glucose, glutamate, succinate, pyruvate and ethanol both in the light and in the dark (Kusmic et al. 1999). Furthermore, Hosotani et al. (1988) reported that a wide range of fatty acids, alcohols and sugars supported considerable growth of *Euglena*. However, assimilation of some of the fatty acids and alcohols was strictly light-dependent. In the case of nitrogen sources, *Euglena* can not use nitrate and urea but it can use ammonia and other kinds of organic nitrogen, and various amino acids can be used as both carbon and nitrogen sources. Thus photoheterotrophic cultivation of *Euglena* using wastewater as the culture medium serves the dual purposes of wastewater treatment and production of useful metabolites. Efficient cultivation of *Euglena* on wastewater, however, requires basic information on the interaction between photoautotrophic and heterotrophic metabolism under photoheterotrophic conditions. Kinetic studies on photoheterotrophic cultures of various photosynthetic cells, such as *Chlorella*, *Spirulina*, *Scenedesmus* and *Haematococcus*, have shown that the growth response depends on both the strain and the light intensity (Endo et al. 1977; Follmann et al. 1978; Ogawa and Aiba 1981; Kobayashi et al. 1992; Marquez et al. 1993). However, there is as yet no detailed study on the kinetics of *E. gracilis* growth in photoheterotrophic cultures.

The aim of this study was therefore to study the effects of light and inorganic carbon supply on the interaction between photoautotrophic and heterotrophic metabolism in photoheterotrophic culture of *E. gracilis*.

Materials and methods

Microorganism and media composition

Euglena gracilis IAM E-6 (strain Z), obtained from the algae collection of the Institute of Applied Microbiology, University of Tokyo, Japan, was used in this study. Hutner medium was modified as described previously (Ogbonna et al. 1998). The glucose concentration in the medium was 10 g l^{-1}.

Cultivation condition

The stock culture (8 ml) was inoculated into 80 ml of the modified Hutner medium in a 100-ml Roux flask. The cells were cultivated under continuous illumination at 30 °C for 24 h. Seven daylight-fluorescent lamps for plant growth experiments (8FL-40-s-PG, National Electric, Tokyo), arranged in parallel on a vertical plane, were used as the light source. The light intensity at the surface of the flask was 100 μmol m^{-2} s^{-1}. Aeration and mixing were achieved by sparging air enriched with 5% CO_2 through a glass-ball filter, which was inserted into the bottom of the Roux flask.

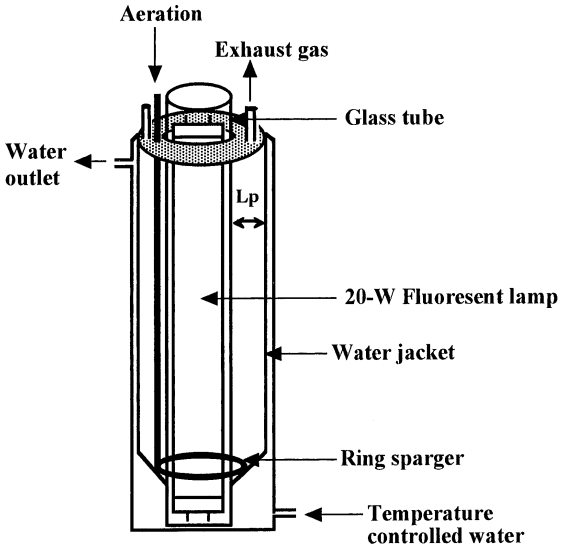

Fig. 1 The internally illuminated photobioreactor used in this study. By varying the light path (LP), as shown in Table 1, three photobioreactors with different light supply coefficients were constructed

The aeration rate was 0.3 volumes of aeration gas per volume of the culture broth per minute (vvm).

Internally illuminated photobioreactors were constructed for studying the effects of light on glucose assimilation, cell growth and α-tocopherol production. Each photobioreactor was internally illuminated by a single 20-W fluorescent lamp. Three photobioreactors with different light supply coefficients were constructed by varying the light path (Fig. 1). The characteristics of the three photobioreactors are summarized in Table 1. The light supply coefficient was defined as the product of the light energy supplied per unit volume (total energy supplied divided by the working volume of the reactor, Et/V) and the light distribution coefficient, Kiv. Detailed information on the calculation of the light supply coefficient was provided elsewhere (Ogbonna et al. 1995). Each reactor was jacketed for temperature control and equipped with a ring sparger. The aeration rate was controlled at 0.3 vvm while the cultivation temperature was controlled at 30 °C by circulating thermostated water through the water jacket. Except in the experiments on the effects of CO_2 concentration, a mixture of 95% air and 5% CO_2 was used for aeration. The cells were cultivated for 48 h. The cell growth and glucose uptake rates were measured after 24 h, while the α-tocopherol concentrations were measured after 48 h.

The effect of light supply coefficient on biomass yield from consumed glucose (Y) was investigated by adding 10 μM of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) to the photoheterotrophic cultures under the three light supply coefficients. The cultures were incubated for 48 h and the yield coefficient was calculated by dividing the cell growth rate (dX/dt) by the glucose consumption rate (dS/dt). Changes in fluorescence intensities of cells during cultivation of *E. gracilis* under various conditions were investigated using 100-ml Roux flasks. The photoautotrophic and photoheterotrophic cultures were illuminated from one surface at a light intensity of 275 μmol m^{-2} s^{-1}, while the heterotrophic

Table 1 Characteristics of the photobioreactors

Et = total light energy supplied (kJ/s), V = working volume (m^3), Kiv = light distribution coefficient (kg/m^3).

Volume (l) (L)	Light path (cm)	Et V^{-1} (kJ m^{-3} s^{-1})	Kiv (kg m^{-3})	Et V^{-1} Kiv (kJ kg m^{-6} s^{-1})
3.80	4.20	0.76	0.22	0.16
2.05	2.76	1.40	0.37	0.52
0.73	1.26	3.94	0.96	3.77

cultures were wrapped with aluminum foil. One milliliter of the culture broth was taken every hour and the fluorescent intensity was measured.

Analysis of the photoautotrophic and heterotrophic growth rates in photoheterotrophic cultures

Assuming that in photoheterotrophic cultures both photoautotrophic and heterotrophic metabolisms proceed concurrently and independently, then the photoheterotrophic growth rate will be the sum of the photoautotrophic and heterotrophic growth rates obtained under similar culture conditions. This can be represented by Eq. 1.

$$dX/dt_{pH} = dX/dt_P + dX/dt_H \quad (1)$$

Here, dX/dt_{PH} is the photoheterotrophic growth rate, dX/dt_P is the cell growth rate under photoautotrophic conditions, and dX/dt_H is the cell growth rate under heterotrophic conditions. However, in cases in which these two metabolic activities interact, the presence of organic carbon source affects the photoautotrophic metabolic activities, and/or light affects the heterotrophic metabolic activities, then the photoheterotrophic growth rate can be modified as shown in Eq. 2. Here $dX/dt_{PH(E)}$ is the experimental total growth rate in photoheterotrophic culture, while β and α are, respectively, the photoautotrophic and heterotrophic interaction constants.

$$dX/dt_{PH(E)} = \beta dX/dt_P + \alpha dX/dt_H \quad (2)$$

The β- and α-values were calculated from Eqs. 3 and 4, respectively, where $dX/dt_{P(PH)}$ is the photoautotrophic growth rate in photoheterotrophic culture and $dX/dt_{H(PH)}$ is the heterotrophic growth rate in photoheterotrophic culture.

$$\beta = \frac{dX/dt_{P(PH)}}{dX/dt_P} \quad (3)$$

$$\alpha = \frac{dX/dt_{H(PH)}}{dX/dt_H} \quad (4)$$

The values of β and α are 1.0 when there is no interaction, more than 1.0 when there is promotion effect and less than 1.0 when there is inhibition effect.

The heterotrophic growth rate in photoautotrophic culture [$dX/dt_{H(PH)}$] was calculated from Eq. 5, where dS/dt is the glucose consumption rate in the photoheterotrophic culture and Y is the biomass yield from the consumed glucose [g dry cell (g glucose)$^{-1}$].

$$dX/dt_{H(PH)} = Y \cdot dS/dt \quad (5)$$

The photoautotrophic growth rate in photoheterotrophic culture was then calculated from Eq. 6.

$$dX/dt_{P(PH)} = dX/dt_{PH(E)} - dX/dt_{H(PH)} \quad (6)$$

Analytical methods

Cell dry weight was determined according to the method described previously (Ogbonna and Tanaka 1996). A glucose test kit (Wako Pure Chemical, Osaka, Japan) was used to measure glucose concentration. Light intensities were measured by an analogue photometer (LI-185B, Licor, Nebraska, USA).

The α-tocopherol content of the cells was determined from chloroform-methanol (1:2 v/v) extracts as described by Shigeoka et al. (1986), using a vitamin-E homologue kit for biochemistry and analysis (Eisai, Tokyo, Japan). Fluorescence intensity was measured using the Plant Efficiency Analyzer (PEA, Hansatech Instruments, England).

All the experiments were done in replicates of either three or four. Analysis of variance (ANOVA) followed by least significance difference (LSD) tests was used to compare the growth rates and α-tocopherol production under various light supply coefficients and carbon dioxide concentrations in the aeration gas. The standard error of the mean (SE) was calculated according to Parker (1980), and the mean values were plotted with 95% confidence limits (average values±SE).

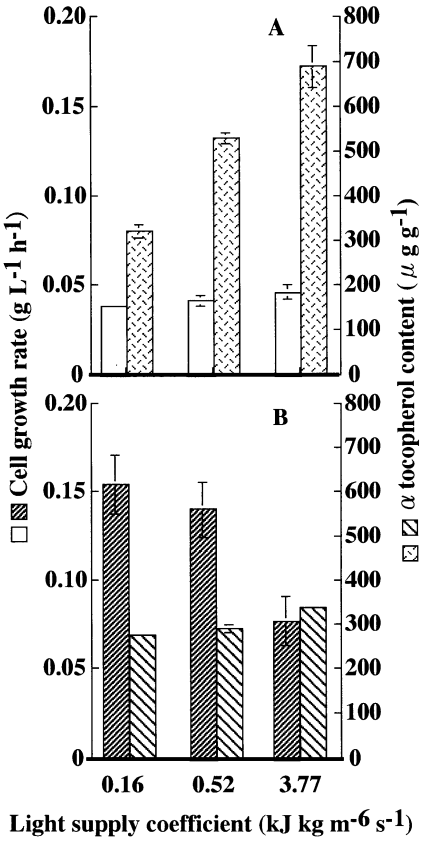

Fig. 2A,B Effects of light supply coefficient on cell growth rate and α-tocopherol content of *Euglena gracilis* cells. **A** Photoautotrophic culture, **B** photoheterotrophic culture

Results

Kinetics of cell growth

The effects of light supply coefficient on cell growth rate in pure photoautotrophic and photoheterotrophic cultures are shown in Fig. 2. Increasing the light supply coefficient from 0.16 to 3.77 kJ kg m^{-6} s^{-1} had no significant effects on cell growth rate in photoautotrophic culture (p=0.05). However, in photoheterotrophic culture, the growth rate decreased sharply at a high light supply coefficient. This implies that photoheterotrophic culture is more sensitive than pure photoautotrophic cultures to photoinhibition.

A comparison of the photoheterotrophic growth rate with the sum of the growth rates obtained in pure heterotrophic and pure photoautotrophic cultures at various light supply coefficients are shown in Fig. 3. At low light supply coefficient, there was no significant difference between the photoheterotrophic growth rate and the sum of the photoautotrophic and heterotrophic growth rates.

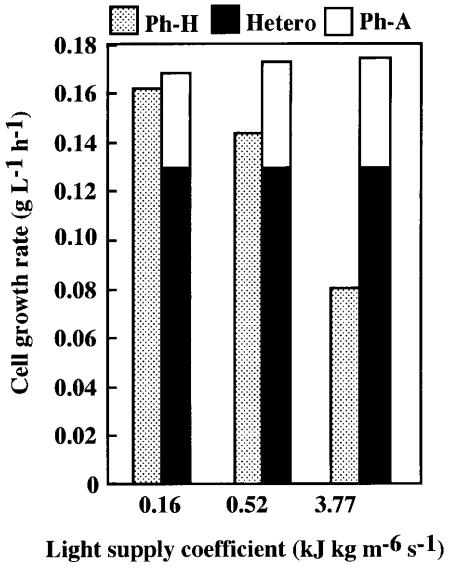

Fig. 3 Comparison of *E. gracilis* cell growth rates in photoheterotrophic cultures under various light supply coefficients with the sums of the corresponding photoautotrophic and heterotrophic growth rates. *Hetero* Heterotrophic culture, *Ph-H* photoheterotrophic culture, *Ph-A* photoautotrophic

This implies that under low light intensities, the photoautotrophic and heterotrophic metabolic activities proceed concurrently and independently. However, at high light supply coefficient, cell growth in the photoheterotrophic culture was inhibited. The photoheterotrophic growth rate was even much lower than that of the heterotrophic growth rate under the same experimental conditions.

Effects of light supply coefficient and carbon dioxide concentration in the aeration gas on heterotrophic and photoautotrophic metabolic activities

The light supply coefficient had no significant effect on the biomass yield from the consumed glucose (p=0.05). The yield (Y) values were the same [0.66±0.025 g-dry cell (g-glucose)$^{-1}$] as observed in heterotrophic culture. The values of $dX/dt_{H(PH)}$ were therefore calculated using a constant Y-value. The effects of light supply coefficient on β- and α-values are shown in Table 2. At low light supply coefficient, the β-value was 1.0 but as the light supply coefficient was increased, it decreased sharply to about 0.3. These results imply that at low light intensity, the presence of glucose and/or heterotrophic metabolism has no significant effect on photoautotrophic growth rate, but photoautotrophic growth is highly inhibited in photoheterotrophic culture under high light intensity. Similarly, the α-value was significantly affected by the light supply coefficient. It was about 0.9 at low light supply coefficient but decreased to less than 0.5 at high light supply coefficient. Thus the sharp decrease in the photoheterotrophic growth rate at high light supply coefficient (Fig. 3) is due to inhibition of both photoautotrophic and heterotrophic metabolic activities.

Table 2 Effect of light supply coefficient on the interaction between heterotrophic and photoautotrophic metabolic activities in photoheterotrophic culture of *Euglena gracilis*

Light supply coefficient (kJ kg m^{-6} s^{-1})	Interaction constants[a]	
	Heterotrophic (α)	Photoautotrophic (β)
0.16	0.898 (0.074)	1.000 (0.055)
0.52	0.843 (0.045)	0.765 (0.062)
3.77	0.495 (0.022)	0.293 (0.027)

[a] Values represent means of four experiments; numbers in parentheses represent the standard error of the means

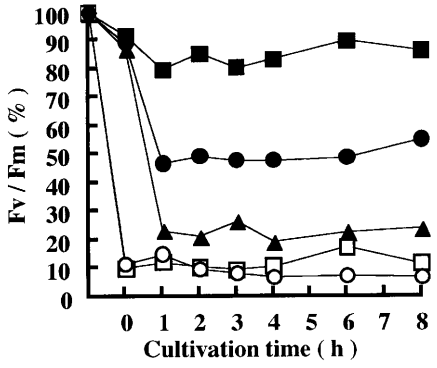

Fig. 4 Changes in fluorescence intensities of cells during cultivation of *E. gracilis* under various conditions. ■ Heterotrophic, ● photoautotrophic, ▲ photoheterotrophic, □ heterotrophic+10 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (*DCMU*), ○ photoautotrophic+10 μM DCMU. The photoautotrophic and photoheterotrophic cultures were done in 100 ml Roux flasks and illuminated from one side at a light intensity of 275 μmol m^{-2} s^{-1}. Fv = variable fluorescence signal, Fm = maximun fluorescence signal

The inhibition of heterotrophic growth was confirmed by measuring glucose uptake by the cells under various light supply coefficients. The specific glucose consumption rate decreased with increase in the light supply coefficient (data not shown). Even at a low light supply coefficient, the specific glucose uptake rate was less than the value obtained under heterotrophic condition. This is consistent with the results shown in Table 2. The α-value was less than 1.0 even at the low light supply coefficient. The fluorescence intensities of the cells grown under various conditions are shown in Fig. 4. As expected, when photosynthesis was inhibited by addition of DCMU either under light or dark condition, the fluorescence of the cells was very low. It is interesting to note that the fluorescence values of the photoheterotrophically grown cells were very close to those of cells cultivated in the presence of DCMU. This confirms that strong light inhibits photoautotrophic growth under photoheterotrophic condition.

A comparison of the cell growth rates and glucose uptake rates under the conditions of heterotrophic, photoheterotrophic and photoheterotrophic in the presence of DCMU is shown in Fig. 5. The glucose consumption and cell growth rates under photoheterotrophic condition in the presence of DCMU were lower than those of the heterotrophic culture. This implies that, even in the absence of pho-

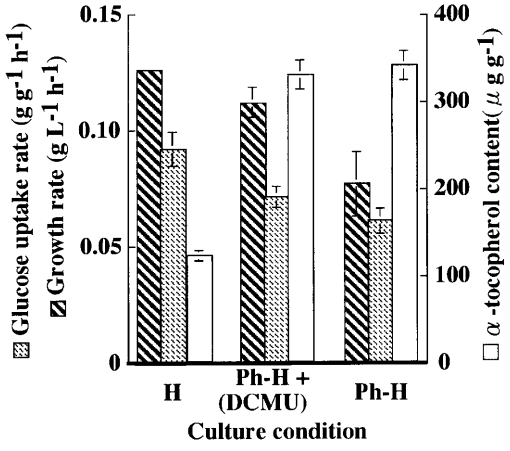

Fig. 5 Effect of DCMU on cell growth rate, glucose uptake rate and α-tocopherol content of the cells in photoheterotrophic culture of *E. gracilis*. *H* Heterotrophic culture, *Ph-H* photoheterotrophic culture. The light supply coefficient was 3.77 kJ kg m^{-6} s^{-1}

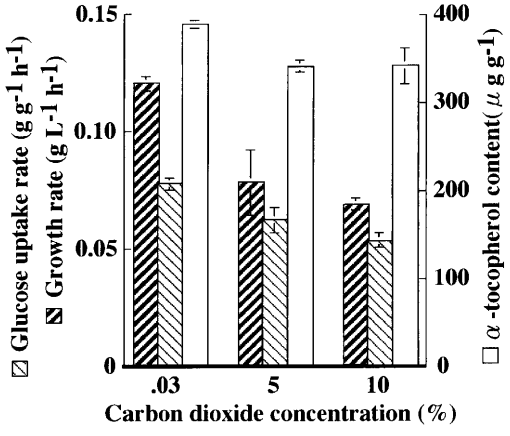

Fig. 6 Effect of carbon dioxide concentration in the aeration gas on cell growth rate, glucose uptake rate and α-tocopherol content of the cells during photoheterotrophic cultivation of *E. gracilis*. The light supply coefficient was 3.77 kJ kg m^{-6} s^{-1}

tosynthesis, high light intensity inhibits glucose metabolism by *E. gracilis*. Furthermore, under photoheterotrophic conditions, the glucose uptake and cell growth rates were lower in the presence than in the absence of DCMU. This implies that, under photoheterotrophic condition, light has both a direct effect (even in the absence of photosynthesis) and an indirect effect (through photosynthesis) on heterotrophic metabolic activities of the cells.

The effects of CO_2 concentration in the aeration gas on cell growth and glucose uptake are shown in Fig. 6. Both the specific glucose uptake rate and the cell growth rate in photoheterotrophic culture were highest when ambient air (0.03% CO_2) was used for aeration and decreased with increasing CO_2 concentration in the aeration gas.

α-Tocopherol synthesis in photoheterotrophic culture

As shown in Fig. 2, although in photoautotrophic culture the cellular content of α-tocopherol increased significantly as the light supply coefficient increased, in the case of photoheterotrophic culture, the effect of the light supply coefficient on cellular α-tocopherol contents was not significant (p=0.05). Compared to heterotrophic culture, α-tocopherol accumulation was higher in photoheterotrophic culture (Fig. 5). Furthermore, even when photosynthesis was inhibited by addition of DCMU, the cellular content of α-tocopherol was almost the same as that obtained in photoheterotrophic culture (Fig. 5). In other words, light stimulates α-tocopherol synthesis even in non-photosynthesizing cells. Although not statistically significant, the α-tocopherol contents of the cells were consistently higher when ambient air was used for aeration than when a mixture of carbon dioxide and air was used (Fig. 6). The same trend was observed even at a low light supply coefficient of 0.16 kJ kg m^{-6} s^{-1} (data not shown).

Discussion

Photoheterotrophic cultures are characterized by the presence of two energy sources, the organic carbon source(s) and light. Assuming that both photosynthesis and organic carbon assimilation can proceed simultaneously, the growth rate is expected to be higher than that achieved under photoautotrophic or heterotrophic conditions. Some reports indicate that under photoheterotrophic condition, photosynthesis and oxidative metabolism of organic substrates proceed independently and in an additive manner so that the growth rate is equal to the sum of the photoautotrophic and heterotrophic growth rates. This has been reported for several strains such as *Chlorella vulgaris* (Ogawa and Aiba 1981; Martinez and Orus 1991), *Chlorella regularis* (Endo et al. 1977), *Haematococcus pluvialis* (Kobayashi et al. 1992) and *Spirulina platensis* (Marquez et al. 1993). Lalucat et al. (1984) reported that with glucose and serine, the photoheterotrophic growth yield of *Chlorella* was even more than the sum of those of the heterotrophic and photoautotrophic cultures. Similarly, Orus et al. (1991) reported that the photoheterotrophic growth rate of *C. vulgaris* UAM 101 surpassed the sum of those of photoautotrophic and chemoheterotrophic cultures.

The independence of photosynthesis and organic carbon assimilation in photoheterotrophic cultures has also been supported by some reports that light does not affect organic carbon assimilation. Martinez and Orus (1991) reported that in *C. vulgaris*, the rates of glucose uptake and oxygen consumption were the same in the dark as in the light (150 μE m^{-2} s^{-1}). They concluded that the glucose uptake system is not photosensitive and mitochondrial respiration is not affected by light. However, most of these experiments were performed under low light intensities. The results of the present investigation show that at low light supply coefficient, the photoheterotrophic growth rate of *E. gracilis* was about equal to the sum of the photoautotrophic and heterotrophic growth rates. Although in the case of *C. vulgaris* (Orus

et al. 1991; Martinez and Orus 1991), organic carbon stimulated cell growth even under light-saturating conditions, some results indicated that the stimulatory effect of organic carbon was observed only under light-limited conditions (Killam and Myers 1956; Follmann et al. 1978; Samejima and Myers 1958; Martinez et al. 1997).

On the other hand, it has also been reported that, even at a moderate light intensity of 6,000 lux, the growth rate of *Scenedesmus acutus* was lower under photoheterotrophic than under photoautotrophic conditions (Ogawa and Aiba 1981). The present study has shown that the growth of *E. gracilis* was highly inhibited under a high light supply coefficient. The decrease in photoheterotrophic growth rate at high light intensities was partly due to photoinhibition of organic carbon assimilation. Decreases in organic carbon assimilation rate at high light intensities have also been reported for *Haematococcus lacustaris* (Moya et al. 1997), *Scenedesmus acutus*, (Ogawa and Aiba 1981), *Chlorella vulgaris* (Follmann et al. 1978), *Chlorella sp* (Lalucat et al. 1984; Kamiya and Kowallik 1987) and *Chlorella pyrenoidosa* (Martinez et al. 1997). Lalucat et al. (1984) noted that, although the specific consumption was lower, glucose was exhausted faster in photoheterotrophic cultures than in heterotrophic cultures. Moya et al. (1997) noted that in *Haematococcus lacustaris*, inhibition of heterotrophic metabolism in photoheterotrophic culture was not due to the direct effect of light but to photosynthetic activity. However, as shown in Fig. 5, in the case of *E. gracilis*, the glucose consumption rate and the cell growth rate were higher in heterotrophic cultures than in photoheterotrophic cultures containing DCMU. The decrease in the photoheterotrophic growth rate of *E. gracilis* under high light intensities was partly due to photoinhibition of photosynthesis (Fig. 4). Previous reports have also shown that photoheterotrophically cultured *Scenedesmus* and *C. vulgaris* had decreased rates of photosynthesis (Ogawa and Aiba 1981; Martinez and Orus 1991). Using isolated chloroplasts and thylakoids from *Euglena*, Landgraf et al. (1997) showed that strong light led to inactivation of stromal enzymes. They stated that light-mediated oxidative stress leads to a specific degradation of the PSII reaction center, D1 protein and ribulose-1,5-bisphosphate carboxylase/oxygenase due to generation of highly toxic oxygen species.

It is important to note that the results of off-line measurement of photosynthetic rates by photoheterotrophically cultured cells may not reflect the rate of photosynthesis in photoheterotrophic cultures. For example, it has been reported that glucose promotes a marked increase of photosynthetic oxygen evolution in photoheterotrophic cultures of *C. vulgaris* at a light intensity of 150 μE m^{-2} s^{-1} (Orus et al. 1991). Glucose stimulation of photosynthesis in *C. vulgaris* UAM 101 was attributed to CO_2 produced from glucose respiration acting as a source of inorganic carbon for photosynthesis. Ogawa and Aiba (1981) noted that the effect of glucose depends on the concentration. Low glucose concentration (5 g l^{-1}) did not have any effect on photosynthetic activity, but at higher glucose concentration (50 g l^{-1}) the rate of photosynthesis decreased.

Another factor that would affect the kinetics of cell growth and nutrient assimilation in photoheterotrophic culture is the inorganic carbon supply. Some of the above reports were done in shake flasks without aeration (external carbon dioxide supply). Carbon dioxide evolved from glucose metabolism is utilized for photosynthesis. However, whether CO_2 from organic carbon assimilation is sufficient to satisfy the demand from photosynthesis (Ogawa and Aiba 1981) or not (Martinez and Orus 1991) would depend on the cell strain and culture conditions. In the present study, the additional supply of CO_2 resulted in a decreased growth rate due to decreased glucose assimilation (Fig. 6).

It is important to note that, unlike in photoautotrophic cultures, the increase in the light supply coefficient in photoheterotrophic cultures did not result in a significant increase in the α-tocopherol content of the cells. In photoheterotrophic cultures, the overall growth rate depends mainly on the heterotrophic metabolic activity (organic carbon assimilation), while α-tocopherol synthesis is controlled by the light availability in the culture. Thus, efficient production of α-tocopherol in photoheterotrophic cultures requires maintaining a balance between heterotrophic and photoautotrophic metabolic activities.

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