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Response of *Spirulina platensis* C1 to High Temperature and High Light Intensity

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ABSTRACT

Response of *Spirulina platensis* strain C1 to high temperature and high light intensity was studied. Specific growth rate of the *S. platensis* C1 cells grown in batch cultures at 35°C with a light intensity of 100 μE/m^2/s was 0.0247 h^{-1}. The specific growth rate decreased to 0.008 h^{-1} at 43°C, which was designated to be the critical temperature for this alga strain C1. A suddenly increasing in light intensity from 100 to 500 μE/m^2/s showed an impact to the cell growth, which it had significant affects to photosynthesis (as O_2-evolution) as well as its pigment content (chlorophyll and phycocyanin). However, a less effect on the alga growth was obtained when the cells were shifted to grow at higher temperature of 43°C. The alga cells grown at high temperature with high light intensity resulted in decreasing protein content while carbohydrate content increased. The fatty acid profiles of the cells grown at high temperature exhibited a decrease in polyunsaturated fatty acid (C18:$3^{\Delta 6,9,12}$) while C18:$2^{\Delta 9,12}$ increased. In contrast, the alga cells grown in a high light intensity showed an increase in C18:$2^{\Delta 9,12}$, while C18:$1^{\Delta 9}$ and total fatty acid content (TFA) were decreased. On the other hand, the combined effects of temperature and light intensity (43°C, 500 μE/m^2/s) showed that the period of recovery in photosynthesis depends upon the period of exposure to the stress parameter.

Key words: *Spirulina platensis*, temperature, light intensity, specific growth rate, photosynthesis

INTRODUCTION

The cyanobacterium *Spirulina platensis* is an oxygenic photosynthetic organism, its biomass is one of a few microalgal products which has been successfully marketed. *Spirulina* is cultivated commercially in several areas of Thailand mainly as health food. This alga is protein-rich and easily digestible. Its valuable chemical components include essential fatty acids and pigments such as carotenes and phycocyanin (Vonshak, 1990). Light intensity and temperature are two parameters that affect the outdoor cultivation since they are largely variable in the natural environment. *Spirulina* has a capability to adjust its photosynthetic processes to adapt to such changes. It has been known that outdoor cultivation of *Spirulina* showed a marked decrease

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in productivity than in laboratory (Vonshak and Richmond, 1985). A possible explanation is a decrease in photosynthetic activity due to photoinhibition under high light intensity. Chanawongse (1992) found that the optimum temperature for *S. platensis* was 35°C. The growth rate of *S. platensis* decreased when temperature increased to 40°C although cells could survive this temperature. In Thailand the outdoor temperature in the summer months can be very high. It has been recorded that the temperature of outdoor ponds in Thailand reached 43°C for a period of time (unpublished data). Thus, this work aimed to study the response of *S. platensis* C1 to high temperature and high light intensity.

MATERIALS AND METHODS

Microorganisms and growth conditions

Spirulina platensis C1, obtained from Prof. Avigad Vonshak (Ben-Gurion University of the Negev, Israel), was cultured in Zarrouk's medium in flasks. Growth temperatures were varied at 35, 37, 39, 41, 43 and 45°C. Cells were grown under cool-white fluorescent lamps providing 100 μE/m^2/s and mixed using a magnetic stirrer. Growth was measured as absorbance at 560 nm for cell density.

Shifting culture conditions

S. platensis C1 was grown in Zarrouk's medium at 35°C under light intensity of 100 μE/m^2/s (control) with continuous stirring for 4 days (optical density at 560 nm reached approximately 1.0). Cells were then shifted to grow at 35°C under 500 μE/m^2/s or at 43°C under 100 μE/m^2/s and incubated for designated periods.

Analytical procedures

Photosynthetic activity was measured by the rate of oxygen evolution using a Clark's type oxygen electrode and calculated as moles O_2-evolved/mg chlorophyll (Chl.)/h (Vonshak *et al.*, 1996).

The concentration of chlorophyll *a* was measured as absorbance at 665 nm after methanolic extraction (Bennett and Bogorad, 1973). Phycocyanin content was estimated after extraction in a phosphate buffer (pH 7) following the procedures of Boussiba and Richmond (1979). Proteins were assayed as described by Lowry *et al.* (1951) after hydrolysis with 1N NaOH and heated in a boiling water-bath for 20 min and the concentration determined by the Folin-Ciocalteau method. Carbohydrate was analyzed using the phenol sulfuric method, and fatty acid was analyzed using a modified method of Lepage and Roy (1984). Fatty acid methyl esters were identified by gas chromatography.

RESULTS

Determination of critical temperature

Specific growth rate of *Spirulina platensis* C1 grown in batch cultures at different temperatures under a light intensity of 100 μE/m^2/s are shown in Figure 1. At optimum temperature (35°C), specific growth rate of the *S. platensis* C1 was 0.0247 h^{-1} and decreased to 0.008 h^{-1} when the alga cells were grown at 43°C. A markedly decrease in chlorophyll *a* was also obtained when cells were grown at 43°C (Figure 2). The chlorophyll contents of the cell grow for

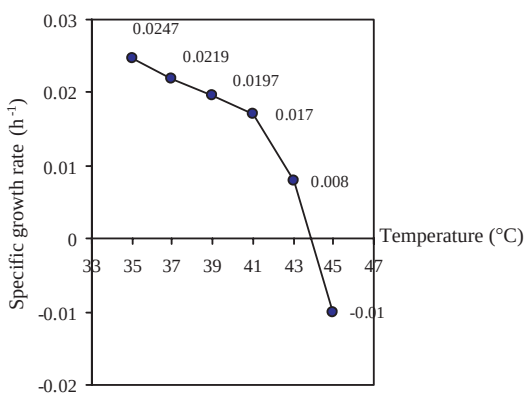

Figure 1 Specific growth rate of *S. platensis* C1 grown at different temperatures.

96 h at 35, 39, 41 and 43°C were 16.7, 8.3, 5.2 and 2.0 mg/l, respectively. The alga cells were declined when temperature increased to 45°C (Figures 1 and 2).

Effect on photosynthetic activity and pigments

The O_2-evolution rate of *S. platensis* C1 showed an average of about 507 μmolO_2/mg Chl./h throughout the study period of 48 h under control cultured conditions at 35°C with light intensity of 100 μE/m^2/s. Phycocyanin content increased from 240 to 338 mg/g cell (about 40%) while chlorophyll increased from 15.2 to 18.7 mg/g cell (about 23%). The photosynthesis as O_2-evolution was remarkably decreased after cells of the cultivar were shifted to high temperature of 43°C with light intensity of 100 μE/m^2/s or 35°C with high light intensity of 500 μE/m^2/s. Figure 3 shows that the photosynthesis rate decreased approximately 50% of the initial rate (500 μmolO_2/mg Chl/h) when cells were shifted to grow at 43°C for 48 h or under 500 μE/m^2/s for 24 h. A previous study of the light saturation curve showed that *S. platensis* C1 has light saturation at 780 μE/m^2/s (Ruengjitchawalya *et al*., 2002) but shifting from 100 μE/m^2/s to 500 μE/m^2/s may cause damage to cell pigments as indicated by the decrease in content of chlorophyll and phycocyanin per cell (Figures 4, 5).

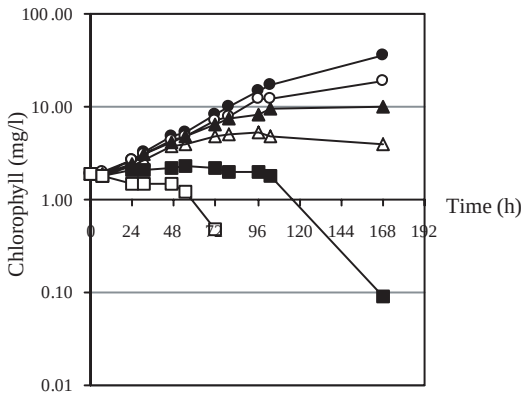

Figure 2 Growth of *S. platensis* C1 at different temperatures (35°C,●; 37°C,○; 39°C,▲; 41°C,△; 43°C,■; 45°C,□).

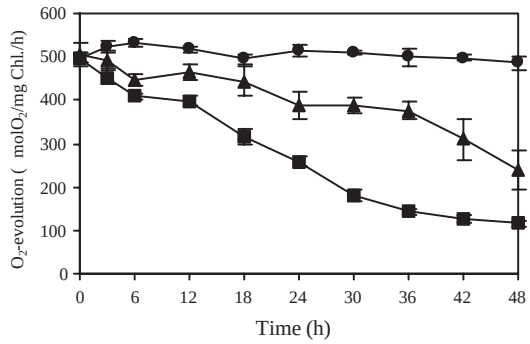

Figure 3 O_2-evolution rate of *S. platensis* C1 grown under various conditions (control,●; 43°C under 100 mE/m^2/s,▲; 35°C under 500 μE/m^2/s,■)

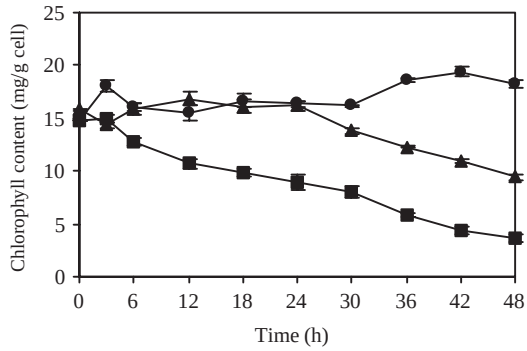

Figure 4 Chlorophyll content of *S. platensis* C1 grown under various conditions (control; ●, 43°C under 100 μE/m^2/s; ▲, 35°C under 500 μE/m^2/s; ■)

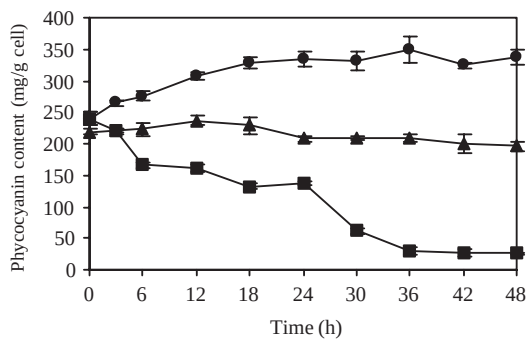

Figure 5 Phycocyanin content of *S. platensis* C1 grown under various conditions (control,●; 43°C under 100 μE/m^2/s,▲; 35°C under 500 μE/m^2/s, ■)

Effects on chemical composition of *S. platensis* strain C1

The *S. platensis* C1 cells that exposed to high light intensity (500 μE/m^2/s) or high temperature (43°C) showed a significant decrease in protein content (about 20-25%), when compared to the cells grown at optimum condition, while a marked increase in carbohydrate content was observed (Table 1). Since the protein of *Spirulina* may contain up to 20% of phycocyanin (Cohen, 1997), reduction of phycocyanin content resulted in a decrease in total protein content. Fatty acid profiles showed that *S. platensis* C1 grown at 43°C exhibited a decrease in polyunsaturated fatty acid (C18:$3^{\Delta 6,9,12}$) while C18:$2^{\Delta 9,12}$ increased. In consistency, *S. platensis* C1 grown under a light intensity of 500 μE/m^2/s exhibited an increase in C18:$2^{\Delta 9,12}$ while C16:$1^{\Delta 9}$ and C18:$1^{\Delta 9}$ including total fatty acid content (TFA) decreased (Table 2).

Photosynthesis recovery

The O_2-evolution rate of *S. platensis* C1 grown at 43°C under 500 μE/m^2/s for 1 h and 3 h decreased about 20% and 49% of the initial rate, respectively. After cells were shifted to grow at optimum condition (35°C under 100 μE/m^2/s; control), O_2-evolution rate of cells from both stressed conditions increased to the same value as control within 4 h and 10 h, respectively. Under outdoor cultivation, temperature and light intensity increase during the morning hours and peak approximately 1 to 3 h resulting in a decrease in photosynthetic activity. However, cells can recover their photosynthetic activity after optimal conditions return in late afternoon. The time needed for recovery depends upon the period of exposure to stress parameter (Figure 6).

DISCUSSION

Decreasing in specific growth rate was observed greater than 70% when temperature increased from 35°C to 43°C. In this study, at temperature of 43°C was designated to be a critical point for growth of *S. platensis* C1, as a sharp drop in the specific growth rate was observed in Figure 1. A similar result has been reported in *S. platensis* strain M2 in which specific growth rate decreased 3 times when the alga cells grown at 42°C (Tomaselli *et al.*, 1988). Phycocyanin and chlorophyll content of *S. platensis* C1 cells grown under control condition increased as growth proceeded. Phycocyanin is a photosynthetic accessory pigment, and an increase in cell concentration resulted in the increase in photosynthetic pigments as a response of cells to

Table 1 Compositions of *S. platensis* C1 grown under various conditions for 48 h.

Conditions (°C, μE/m^2/s)	Protein (% of dry weight)	Carbohydrate (% of dry weight)
35, 100 (control)	57 ± 2	23 ± 1
35, 500	42 ± 2	42 ± 1
43, 100	47 ± 1	36 ± 2

Table 2 Fatty acid composition of *S. platensis* C1 grown under various conditions for 48 h.

Conditions	Fatty acid composition (% TFA)						TFA
(°C, μE/m^2/s)	16:0	16:1	18:0	18:1	18:2	18:3	(%dw)
35, 100 (control)	45.9	5.1	1.1	9.1	24.7	14.2	4.11
35, 500	45.5	4.3	2.7	6.2	28.0	13.4	2.04
43, 100	46.1	4.0	3.9	7.5	25.8	12.6	3.66

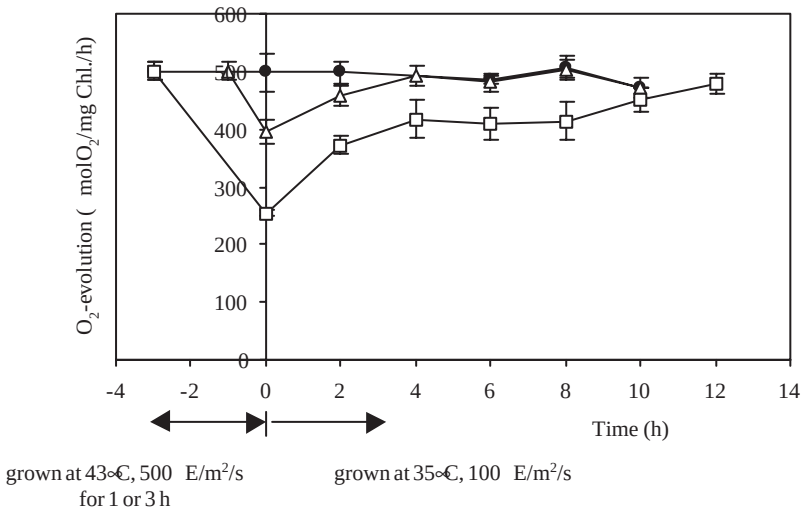

Figure 6 O_2-evolution of *S. platensis* C1 after cells were grown under stress conditions (control,●; 43°C under 500 μE/m^2/s for 1h,△; 43°C under 500 μE/m^2/s for 3h,□)

enhance the light entrapment capability**.** However, high light intensity (500 μE/m^2/s) causes damage to pigments of *S. platensis* C1. Hihara *et al.* (2001) reported that *psb*B-gene encoding CP47 (chlorophyll protein complexes of 47 kDa) and *psd*C which encodes CP43 (chlorophyll protein complexes of 43 kDa) decreased within the first period of exposure to high light (300 μE/m^2/s). Transcription of *cpc* genes which are related to phycocyanin decreased 20-fold within 1 h of high light exposure and resumed to the level of 50% of the initial level after 15 h. The *cpc* genes may be downregulated more strictly than *apc* genes (related to allophycocyanin), during exposure to high light because phycocyanin, which is located at the terminal region of the phycobillisome rods, is the primary target for the reduction of antenna size. Moreover, our study corresponded to Nomsawai *et al.* (1999) who reported that the 33kDa linker polypeptide disappeared under high light (500 μE/m^2/s) resulting in smaller phycobilisomes, and that the decrease in the number of phycobilisomes correlated well with the decrease in phycocyanin and allophycocyanin content from cells that were shifted from low (50 μE/m^2/s) to high light. Also, study of *Synechocystis* found that almost all of the photosystem I (PSI) genes were downregulated by five- to ten-fold within 1 h of the transfer from low (20 μE/m^2/s) to high light (300 μE/m^2/s). Notably, the level of *psa*AB transcripts for the reaction center of PSI declined by 30-fold of its original value after 1 h of high light treatment. In addition, transcripts of genes involved in the oxygen-evolving complex, *psb*O, *psb*U and *psb*V, were all downregulated to half the level in low light within 15 min (Hihara *et al*., 2001). In cyanobacteria, the PSII/PSI ratio generally increases upon the shift to high light (Kawamura *et al*., 1979). Declining PSI content would be expected to lower the susceptibility of the cells to high light damage particularly under prolonged exposure (Hihara and Lkeuchi, 1998). Simultaneously, phycobillisome size and photosystem content probably were reduced to avoid adsorption of excess light energy.

At high temperature (43°C), *S. platensis* C1 showed an increase in C18:2 while C18:3 decreased. These results correlated with data obtained in *S. platensis* M2 that a decrease in polyunsaturated fatty acids of cell was found at

high temperature (Tomaselli *et al*., 1988). Thus, at temperatures above the optimum, the fatty acids showed a lower degree of unsaturation because C18:3 biosynthesis becomes progressively blocked resulting in the accumulation of C18:2. Besides, an increase in a neutral lipid, C18:0, was observed. In addition, Deshnium *et al*. (2000) found that at the transcriptional level, the expression of *desC*, *desA*, and *desD* genes (encoding Δ9, Δ12, and Δ6 desaturase, respectively) were not affected by the temperature shift to 40°C, however, the degradation rate of *desD* mRNA increased when the cells were incubated at 40°C.

An increase in C18:$2^{\Delta 9,12}$ of *S. platensis* C1 grown under high light intensity was also observed. Kis *et al*. (1998) reported that *desA* and *desD* were strongly induced by light. In the same way, Hihara *et al*. (2001) found that *desA* and *desD* of *Synechocystis* cells were induced within 15 min of exposure to high light (300 μE/m^2/s) whereas *des*C was constitutive.

CONCLUSIONS

Specific growth rate of *S. platensis* C1 grown at various temperatures above 35°C were found to progressively decrease as temperatures increased. *S. platensis* C1 responded to high temperature and high light intensity by decreasing photosynthetic activity, photosynthetic pigments and protein. High light intensity at 500 μE/m^2/s showed more effects on photosynthesis and its pigment than high temperature (43°C). Both high temperature and high light intensity similarly decreased the protein content while carbohydrate increased. Changes in fatty acid profiles of *S. platensis* C1 depends on growth conditions, and a remarkable increase in C18:$2^{\Delta 9,12}$ was observed when cells were grown under high light intensity.

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