



Short Communication

Characterization of the thermal-tolerant mutants of *Chlorella* sp. with high growth rate and application in outdoor photobioreactor cultivation

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ABSTRACT

In this study, two thermal-tolerant mutants of *Chlorella* sp. MT-7 and MT-15, were isolated. In indoor cultivation, specific growth rate (μ , d^{-1}) of the mutants were 1.4 to 1.8-fold at 25 °C and 3.3 to 6.7-fold at 40 °C higher than those of wild type. The carbon dioxide fixation rate of both microalgal mutants was also significantly higher than that of wild type. In outdoor closed cultivation, where the temperature of culture broth was 41 ± 1 °C, the μ of mutant strain MT-15 was $0.238 d^{-1}$ during an 8-day cultivation. Whereas, the growth of wild type was inhibited in the outdoor cultivation. Our results show that the isolated microalgal strains are adaptable to be applied in outdoor cultivation in subtropical zones.

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1. Introduction

Global warming, which has been a concern in world-wide, is due to the increasing carbon dioxide (CO₂) level in atmosphere. The global atmospheric concentration of CO₂ has increased from a pre-industrial value of about 280–379 ppm in 2005. Microalgae have very efficient photosynthesis, grow faster than other plants and are able to convert CO₂ to biomass efficiently. In recent years, biological CO₂ fixation using microalgal photosynthesis has emerged as a potential option because of its effectiveness and economical in CO₂ reduction. Among the microalgae which were studied in CO₂ fixation, *Chlorella* sp. has been used in many studies and shows high CO₂ fixation rate (Cheng et al., 2006; Chiu et al., 2009b; de Morais and Costa, 2007).

During outdoor cultivation with solar as the light source, biomass productivity is strongly affected by environmental factors such as irradiation and temperature (Ugwu et al., 2007). The temperature of microalgal culture broth in photobioreactors can increase to about 40 °C by irradiation of sunlight in subtropical zones. The microalgal growth would be highly inhibited at such high temperature if the cultivation is not provided with cooling system. Thermal-tolerant species could grow well under high temperature and would significantly reduce the cooling costs (Ono and Cuello, 2007).

Many thermal-tolerant microalgal strains have been isolated from hot springs (Hsueh et al., 2007; de Bashan et al., 2008). However, it is time consuming to purify the cultures from other microorganisms. In the present study, two thermal-tolerant mutant strains of *Chlorella* sp. were isolated by mutagenic chemical treatment. The growth pattern, CO₂ fixation rate and lipid content of the microalgae were determined for characterizations of the isolated *Chlorella* sp. mutant strains.

2. Methods

The microalga (wild type) *Chlorella* sp. was obtained from Taiwan Fisheries Research Institute, Tung-Kang, Taiwan. The microalga was cultured in artificial sea water in each batch culture with the medium which has the composition (per liter) of 750 mg NaNO₃, 44.11 mg NaH₂PO₄·H₂O, 43.6 mg Na₂-EDTA, 31.6 mg FeCl₃·6H₂O and micronutrients (trace elemental solution) including 1.8 mg MnCl₂·4H₂O, 0.1 mg CoCl₂·6H₂O, 0.1 mg CuSO₄·5H₂O, 0.23 mg ZnSO₄·7H₂O, 0.06 mg Na₂MoO₄, 1 mg vitamin B₁, 5 µg vitamin B₁₂ and 5 µg biotin (Chiu et al., 2009b).

The wild type cells were mutagenized following the method described by Chaturvedi et al. (Chaturvedi and Fujita, 2006) with some modifications. About 1×10^7 cells of *Chlorella* sp. were treated with 100 mM ethyl methane sulfonate (EMS) for 1 h, and each approximate 1×10^3 cells were plated on agar plates and were incubated at 40 °C. The bigger colonies were selected and cultivated in indoor vertical bubble column photobioreactor (cultured volume is 4 L; Chiu et al., 2009b) subsequently. The growth rate of the mutant

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strains were compared with the wild type at the cultivation temperatures of 25, 30, 35 and 40 °C. All the cultures were aerated with 5% (v/v) CO₂ continuously at 0.25 vvm (volume gas per volume media per min) and supplied with light intensity of 300 μmol m⁻² s⁻¹. The microalgal growth based on the biomass concentration (g L⁻¹) was determined by spectrophotometric method (Chiu et al., 2008). The specific growth rate (μ, d⁻¹) was measured during the exponential growth of microalgae (Ono and Cuello, 2007). For the analysis of lipid content, microalgal lipid was determined according to the method reported by Chiu et al. (2009a).

The growth experiment of semicontinuous cultivation was performed when the biomass concentration in the batch cultures reached to about 1 g L⁻¹ (OD₆₈₂ = 5). In the semicontinuous cultivation, half of the culture broth was replaced and 0.5-fold of medium was added each day.

The CO₂ fixation rate of the microalgal cultures was determined when the cultures were grown at 40 °C by the semicontinuous cultivation with an influent of 5% CO₂ at 0.25 and 0.5 vvm. The CO₂ concentration in airstreams, i.e., CO_{2(g)}, was measured using a Guardian Plus Infrared CO₂ Monitor D-500 with a sensitivity of 0.05% of CO_{2(g)} (Edinburgh Instruments, Livingston, UK). The CO₂ fixation rate (mg min⁻¹) was determined as, $R_{CO_2} = [(P_0 + \rho gh)y_{CO_2in} - P_0 y_{CO_2out}] F_{air} M_{wCO_2} / 8.314 TV_{culture}$ in which, y_{CO_2in} presents the amount of CO₂ influent (%), y_{CO_2out} presents the amount of CO₂ effluent (%), P_0 is atmospheric pressure (Pa), ρ is density of liquid (kg m⁻³), h is vertical distance of culture medium (m), F_{air} is gas flow rate (L min⁻¹), M_{wCO_2} is molecular weight of CO₂ (g mol⁻¹), T is absolute temperature (K), and $V_{culture}$ is volume of culture medium (L) (Cheng et al., 2006).

The mutant strain which was the most tolerant to the high temperature of 40 °C was cultivated in a large scale of outdoor closed and vertical bubble column photobioreactor (cultured volume is

40 L). The experiment was carried out over a period of 8-day during the summer of 2008. The cultures were supplied with 5% (v/v) CO₂ and 0.25 vvm aeration rate. The photosynthetic photon flux at the photobioreactor location during the day time was averagely 1500 μmol m⁻² s⁻¹. A nighttime light supply of 300 μmol m⁻² s⁻¹ was also established.

A Student's *t*-test was used to evaluate differences between groups of discrete variables. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

Fig. 1 demonstrates that the μ of *Chlorella* sp. mutant MT-7 and MT-15 in indoor cultivation were 1.4- and 1.8-fold at 25 °C and 3.3- and 6.7-fold at 40 °C cultivation compared with those of the wild type during an 8-day cultivation, respectively. The wild type cultures did grow poor at 35 and 40 °C. The mutant strains showed the maximum μ at 30 °C cultivation and moderate growth at 35 and 40 °C. The optimal temperature for most microalgal species was in a range of 22–28 °C. This was confirmed that 25 °C was the optimal temperature for growth of the wild type *Chlorella* sp. in this study. Our results indicate that the microalgal mutants MT-7 and MT-15 are thermal-tolerant and have high growth potential. It is identified that the growth and thermal-tolerant potential of the mutated microalgal strains selected in the present study are comparable to those of microalgal strains isolated from the nature (de Bashan et al., 2008; Ono and Cuello, 2007).

Table 1 demonstrates the lipid contents of wild type, MT-7 and MT-15 cultivated at 25, 30, 35 and 40 °C. The average lipid content of both mutant strains was lower than wild type across all of the cultivation temperatures. The lipid content of wild type in stationary phase of cultivation was significantly higher ($p < 0.05$) than that in exponential phase at 25, 30 and 35 °C. But, there was no effect of the growth phase on the lipid accumulation in both mutant strains when the cultivation temperatures were ranging from 25 to 40 °C, except MT-15 cultivated at 25 °C. Overall, the mutant strains were not able to accumulate lipid efficiently compared to the wild type. However, the mutant strains isolated in this study grew faster than the wild type strain and were tolerant at higher temperature. Therefore, when the microalgal cells cultured at 35 and 40 °C, but not at 25 and 30 °C, the daily lipid productivities of the mutant strains at higher temperature (35 and 40 °C) were greater than the wild type.

In the semicontinuous cultivation at 25 °C, the growth of mutant strains was faster than that of wild type. The growth of MT-7, MT-15 and wild type was maintained consistently at biomass concentration from 0.8 to 1.6 g L⁻¹, 0.9 to 1.8 g L⁻¹ and 0.45 to 0.90 g L⁻¹, respectively, each day during an 8-day cultivation (Fig. 2a). In the semicontinuous cultivation at 40 °C, the wild type culture was grown from biomass concentration about 0.4 g L⁻¹ on the first day of semicontinuous cultivation. During an 8-day semicontinuous culture, the biomass concentration of wild type culture gradually decreased. However, MT-7 and MT-15 strain grew from

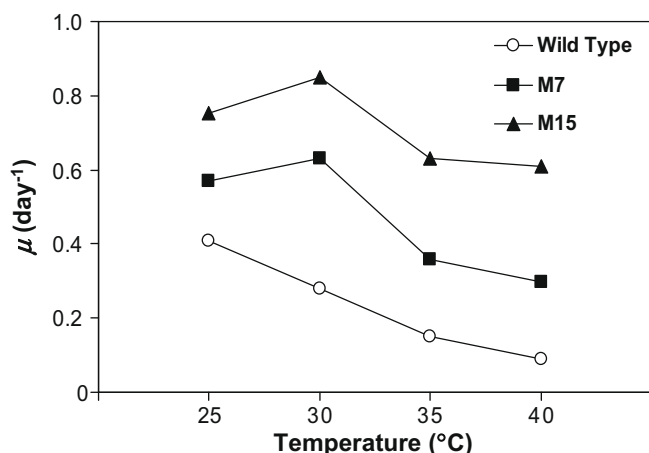


Fig. 1. Specific growth rate (μ, d⁻¹) of *Chlorella* sp. wild type and mutant strain MT-7 as well as MT-15 at cultivation temperature of 25, 30, 35 and 40 °C.

Table 1

Effect of growth phase and temperature on lipid content (%) of wild type, mutant strain MT-7 and MT-15.

Strain	25°C		30°C		35°C		40°C	
	Exponential ^a	Stationary ^b	Exponential	Stationary	Exponential	Stationary	Exponential	Stationary
Wild type	12.3 ± 0.5	22.5 ± 0.9*	12.5 ± 0.7	17.3 ± 1.7*	14.7 ± 1.7	19.4 ± 2.3*	14.1 ± 0.6	16.2 ± 0.6
MT-7	12.8 ± 1.5	11.5 ± 0.9	10.3 ± 1.7	9.9 ± 1.0	12.6 ± 0.6	12.9 ± 2.5	12.4 ± 0.8	12.0 ± 0.7
MT-15	9.2 ± 1.1	13.8 ± 0.6*	10.4 ± 0.6	11.5 ± 0.7	11.7 ± 0.7	9.6 ± 0.7	11.8 ± 0.5	8.8 ± 0.5*

^a Sample was collected during the exponential phase after 3 days of cultivation.

^b Sample was collected during the stationary phase after 13 days of cultivation.

* There were significant differences ($p < 0.05$) in the lipid content of the culture collected at stationary phase compared to the exponential phase for each strain at each cultivation temperature. Each data indicates the mean ± SD, which was measured from three independent cultures.

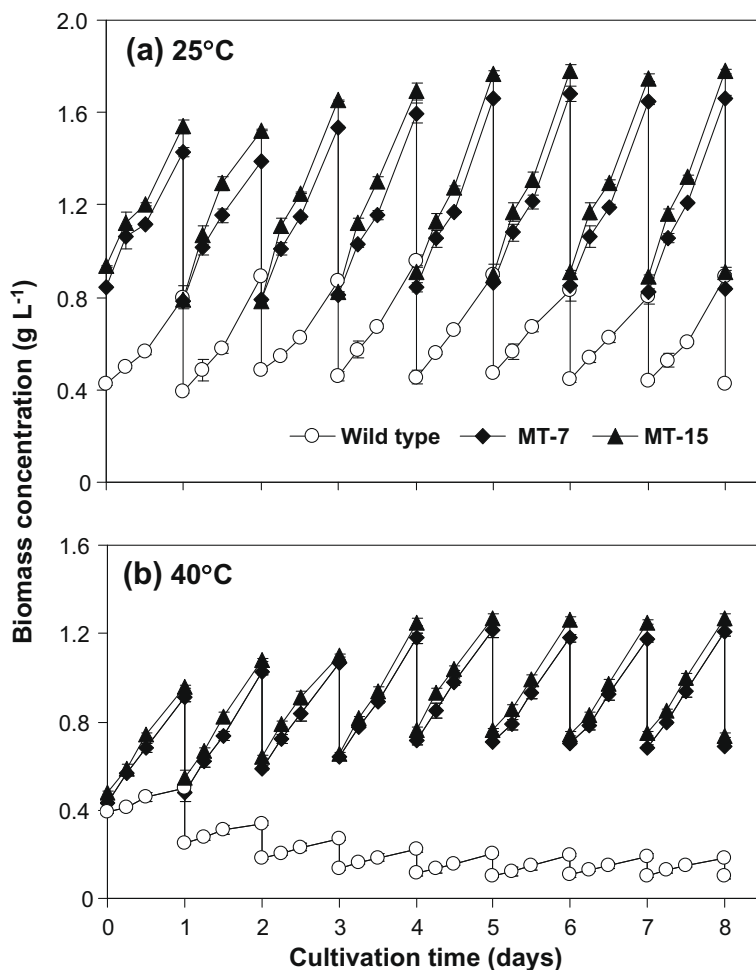


Fig. 2. Growth profiles of *Chlorella* sp. mutant strain MT-7 and MT-15 compared to wild type in the semicontinuous cultivation with 5% CO₂ aeration at (a) 25 °C and (b) 40 °C.

Table 2

The CO₂ fixation rate of wild type, mutant strain MT-7 and MT-15 for the cultivation at 40 °C with 5% CO₂ aeration.

Aeration rate (vvm) ^a	Biomass concentration (g L ⁻¹)	CO ₂ fixation rate (R_{CO_2} , mg min ⁻¹)		
		Wild type	MT-7	MT-15
0.25	1	11.80 ± 0.10 ^b	12.45 ± 0.47	10.92 ± 0.23
	2	ND ^c	14.43 ± 0.16	14.86 ± 0.20
	3	ND	16.89 ± 0.19	17.72 ± 0.19
0.5	1	15.52 ± 0.11	18.24 ± 1.03	17.13 ± 0.24
	2	ND	19.43 ± 0.76	21.38 ± 0.77
	3	ND	21.14 ± 0.70	25.65 ± 1.03

^a The aeration rates of CO₂ at 0.25 vvm and 0.5 vvm are equal to 89.43 mg min⁻¹ and 178.86 mg min⁻¹ CO₂, respectively.

^b Each data indicates the mean ± SD, which was measured from three independent cultures.

^c ND, not detected. The wild type cultures would not reach to the biomass concentration of 2 g L⁻¹ and 3 g L⁻¹ at the cultivated condition. Therefore, the R_{CO_2} could not be detected.

0.4 to 0.9 g L⁻¹ on the first day, and the growth was stable and maintaining from 0.6 to 1.2 g L⁻¹ from the third day onward, after which half of the culture broth was replaced with fresh medium daily (Fig. 2b). In short, the mutant strains, but not the wild type strain, were able to maintain growth potential at an environmental temperature of 40 °C.

The CO₂ fixation rate of the microalgae cultured in a semicontinuous cultivation was determined when the microalgal strains

were grown to the biomass concentration of 1, 2 or 3 g L⁻¹ at 40 °C (Table 2). The aerated CO₂ concentration was 5% and the aeration rate was set as 0.25 and 0.5 vvm. Overall, the results show that there was significant higher of CO₂ fixation rate at 0.25 vvm compared to 0.5 vvm CO₂ aeration rate in all the cultures. In addition, significant higher of CO₂ fixation rate was found at higher biomass concentration compared to lower biomass concentration (i.e., 3 > 2 > 1 g L⁻¹) in all the cultures.

The fixation rate of CO₂ increased when the biomass concentration of cultures increases. This is the reason that more CO₂ was captured by the microalgae to maintain the growth at a higher biomass concentration. High density cultures result in higher viscosity which subsequently increase gas retention time for CO₂ absorption and therefore enhance the CO₂ removal efficiency (Chiu et al., 2008). Significant higher of CO₂ fixation rate was also found when the aeration rate decreases in the result of the increasing of CO₂ absorption from bubbling gas. This is caused by the increase of surface area per unit gas volume of the bubble which will also enhance the CO₂ removal efficiency (Chiu et al., 2009b).

Chlorella sp. MT-15 was selected and applied for the cultivation in an outdoor closed photobioreactor as it was the faster-growing strain cultivated at 40 °C. The microalgal culture used sunlight for photosynthesis in the day, and artificial light with intensity of 300 μmol m⁻² s⁻¹ was supplied to the surface of the photobioreactor at night. During the experimental period of 8 days, the average air temperature was 29 ± 2 °C, while the average culture broth temperature was 41 ± 1 °C. The wild type culture did not seem

growing; however, MT-15 grew well with $\mu = 0.238 \text{ d}^{-1}$. Nighttime growth delays were not observed because the cultures were supplied with artificial light. The maximum volumetric productivity obtained from MT-15 cultivated up to 40 °C in batch culture in this study, was $0.35 \text{ g L}^{-1} \text{ d}^{-1}$. During the summer in subtropical zones, study of outdoor microalgal cultivation was mostly supplied with cooling system by circulating thermostatic water through the photobioreactors (Chini Zittelli et al., 2006). However, in this study, the cultures of mutant strain could grow well at the environmental temperature reaching 40 °C without any operation to lower the temperature.

4. Conclusion

The mutant strains *Chlorella* sp. MT-7 and MT-15 isolated in this study are thermal-tolerant, grow fast with a high density (i.e., high biomass concentration) at temperature of 40 °C, and could capture CO₂ with a significantly high efficiency compared to their wild type. In addition, MT-7 and especially MT-15 have the potential to be applied at outdoor cultivation in subtropical region without cooling system, thereby reducing the cost of outdoor cultivation.

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