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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.

1. Introduction

Global warming, which has been a concern in world-wide, is due to the increasing carbon dioxide (CO_2) level in atmosphere. The global atmospheric concentration of CO_2 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 CO_2 to biomass efficiently. In recent years, biological CO_2 fixation using microalgal photosynthesis has emerged as a potential option because of its effectiveness and economical in CO_2 reduction. Among the microalgae which were studied in CO_2 fixation, *Chlorella* sp. has been used in many studies and shows high CO_2 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, CO_2 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 $NaNO_3$, 44.11 mg $NaH_2PO_4{\cdot}H_2O$, 43.6 mg $Na_2{\cdot}EDTA$, 31.6 mg $FeCl_3{\cdot}6H_2O$ and micronutrients (trace elemental solution) including 1.8 mg $MnCl_2{\cdot}4H_2O$, 0.1 mg $CoCl_2{\cdot}6H_2O$, 0.1 mg $CuSO_4{\cdot}5H_2O$, 0.23 mg $ZnSO_4{\cdot}7H_2O$, 0.06 mg Na_2MoO_4, 1 mg vitamin B_1, 5 μg vitamin B_{12} 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) CO_2 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} (OD_{682} = 5). In the semicontinous cultivation, half of the culture broth was replaced and 0.5-fold of medium was added each day.

The CO_2 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% CO_2 at 0.25 and 0.5 vvm. The CO_2 concentration in airstreams, i.e., $CO_{2(g)}$, was measured using a Guardian Plus Infrared CO_2 Monitor D-500 with a sensitivity of 0.05% of $CO_{2(g)}$ (Edinburgh Instruments, Livingston, UK). The CO_2 fixation rate (mg min^{-1}) was determined as, $R_{CO_2} = [(P_0 + \rho gh)y_{CO_2 in} - P_0 y_{CO_2 out}]F_{air}M_{wCO_2}/8.314\,TV_{culture}$ in which, $y_{CO_2 in}$ presents the amount of CO_2 influent (%), $y_{CO_2 out}$ presents the amount of CO_2 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 CO_2 (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) CO_2 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.

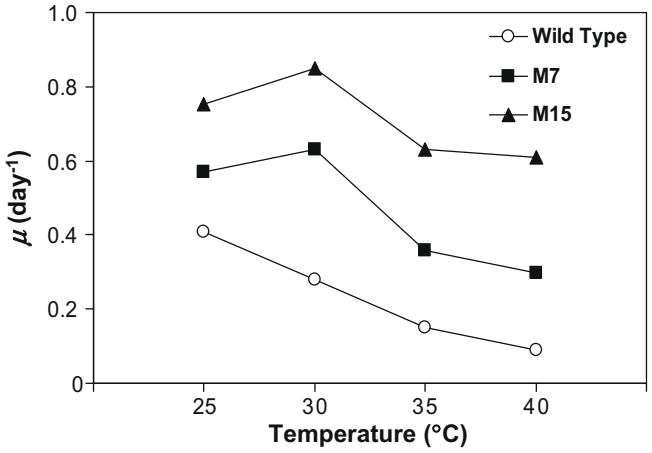

Fig. 1. Specific growth rate (μ, d^{-1}) of *Chlorella* sp. wild type and mutant strain MT-7 as well as MT-15 at cultivation temperature of 25, 30, 35 and 40 °C.

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

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.