Bioresource Technology 136 (2013) 360–367

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Increased lipid productivity and TAG content in *Nannochloropsis* by heavy-ion irradiation mutagenesis

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HIGHLIGHTS

- Heavy ion irradiation mutagenesis was firstly applied in microalgae breeding.
- The mutant HP-1 showed higher biomass production than the wild type *Nannochloropsis*.
- Its lipid productivity was increased by 28% compared to the wild type.
- The reason caused its higher biomass accumulation and lipid productivity was studied.
- Further, its TAG content was higher and polar lipid was lower than the wild type.

ARTICLE INFO

Article history:
Received 6 October 2012
Received in revised form 4 March 2013
Accepted 6 March 2013
Available online 14 March 2013

Keywords:
Microalgae
Mutation breeding
Strain selection
Biodiesel

ABSTRACT

One mutant (HP-1) with higher growth rate was obtained from *Nannochloropsis oceanica* IMET1 by heavy-ion irradiation mutagenesis. Compared to the wild type, the biomass accumulation and maximum growth rate of HP-1 were individually increased by 19% and 6%, and its lipid productivity was increased by 28% from 211 to 271 mg L^{-1} d^{-1}. Subsequently analysis indicated photosynthetic efficiency of HP-1 was higher than that of wild type during cultivation. Further, lipid composition analysis indicated TAG content of HP-1 was 14% higher, while polar lipid content was 15% lower than that of wild type. Moreover, fatty acid profiles analysis revealed no significant variation was found between the two strains. The mutant is discussed in terms of its comparative advantage over the wild type with respect to its potential utilization for biodiesel production. Owing to its higher lipid productivity and TAG content, HP-1 could be considered as a valuable candidate for microalgal biodiesel production.

1. Introduction

Due to anthropogenic climate change result from carbon emissions and reduction in the remaining easily exploitable fossil fuel, renewable biofuels especially biodiesel have received much attention worldwide in recent years. Microalgae have been considered as one of the best feedstock for biodiesel production due to their characteristics, such as high growth rate, high triacylglycerol (TAG) content, the major feedstock for biodiesel production, and no compete for land with crops used for food production (Ahmad et al., 2011; Chisti, 2007). However, microalgal biodiesel has not been widely commercialized due to its high cost that results from many factors, including low lipid productivity, highly energy-consuming harvesting technologies and expansive oil extraction (Zhang et al., 2010; Hu et al., 2006). Much work is needed to reduce the cost of microalgal biodiesel production in the upstream and downstream of total biodiesel production.

To increase lipid productivity in microalgae, strain improvement by induced cell mutagenesis and mutant selection has been suggested as an effective method (Anandarajah et al., 2012). In general, mutagens fall into two main categories in microalgae breeding, including physical (e.g. UV-, γ- and X-rays) and chemical method [e.g. ethyl methane sulfonate (EMS) nitrosomethyl guanidine. Heavy-ion beams, possessing the higher relative biological effect compared to X- and γ-rays, are expected to have a wide mutation spectrum and increased mutation frequency, and have been used effectively as a breeding method in plants and microorganisms (Kazama et al., 2011; Wang et al., 2009). To the best of our knowledge, little has been reported using this effective method in microalgae breeding.

At present, only a few microalgae are being considered or are already being exploited for biodiesel production. Marine-derived *Nannochloropsis* is one of them (Doan et al., 2011; Umdu et al., 2009; Koberg et al., 2011; Li et al., 2011; Chen et al., 2012). With

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http://dx.doi.org/10.1016/j.biortech.2013.03.020

the decreasing of the fresh water resource, marine-derived microalgae have advantage over other microalgae in the future large-scale culture for commercialized microalgal biodiesel. *Nannochloropsis* is a marine unicellular alga belonging to the Eustimatophyceae which grows fast, typically doubling their biomass once a day, and also possesses high oil productivity (Anandarajah et al., 2012). *Nannochloropsis* has been identified as a promising feedstock for biodiesel production in recent years (Umdu et al., 2009; Koberg et al., 2011; Li et al., 2011; Chen et al., 2012). Recently, strain improvement of *Nannochloropsis* for biodiesel production by chemical method (EMS) has been reported (Anandarajah et al., 2012; Doan and Obbard, 2012). However, physical method to improve its characteristics for biodiesel production has never been reported.

In this study, heavy-ion irradiation mutagenesis was applied in the wild type *Nannochloropsis oceanica* IMET1 to improve its characteristics for biodiesel production. This is the first report by using heavy-ion irradiation mutagenesis in the microalgae breeding. This work reported here is also a first attempt to increase lipid productivity with the use of physically induced mutant produced from wild type *Nannochloropsis*. Herein, we reported the heavy-ion irradiation mutagenesis method of wild type *N. oceanica* IMET1. After mutation screening, one mutant (HP-1) was obtained with higher growth rate, and then its biomass and lipid accumulation were investigated. Moreover, the reason caused high biomass and lipid production, as well as lipid characteristics involved in the biodiesel production were also analyzed in the HP-1 mutant.

2. Methods

2.1. Organism and culture condition

N. oceanica IMET1 was kindly provided by Dr. Feng Chen from The University of Maryland. The strain was grown in seawater, supplemented with BG-11 medium, and maintained at 25 ± 1 °C under continuous illumination provided by daylight fluorescent tubes at 80–100 μmol photons m^{-2} s^{-1}.

2.2. Heavy-ion irradiation mutagenesis

Cell suspensions of *N. oceanica* IMET1 in early exponential growth phase at 1.5×10^7 cells/mL were used for mutagenesis. Carbon ion beam was provided by the Heavy-Ion Research Facility in Lanzhou (HIRFL), Institute of Modern Physics, Chinese Academy of Sciences. The carbon ion energy was 80 MeV/μ. The average linear energy transfer values (LET; the energy transferred per unit length, keV μm^{-1}) was 31 keV μm^{-1}. The algae cells were irradiated by carbon ions with different doses, including 20, 40, 60, 80, 100, 120, 140 and 160 Gy referenced from the previous reports (Kazama et al., 2011; Wang et al., 2009). Cells from each treatment were kept in darkness overnight prior to determination of cell mortality. Cell mortality was determined by plating approximately 500 untreated microalgae cells and identical dilution of the treated cells on agar plates in triplicate. In addition, approximate 1000 cells from each treatment condition were plated on agar plates, and then cultured at 25 °C under continuous illumination at 50 μmol photons m^{-2} s^{-1} until the algae colonies emerged. Approximately 3000 colonies from above 50% mortality were selected for mutation screening. After large-scale screening, one mutant (HP-1) was obtained with higher growth rate.

2.3. Microalgal growth property

The HP-1 mutant and wild type were inoculated to 400 mL bubble column bioreactor (40 mm diameter, 600 mm length) with sterile gas composed of air supplemented with 2% (V/V) CO_2. The average aeration rate was 0.2 vvm. The microalgae was cultured at 25 °C under continuous illumination at 100 μmol photons m^{-2} - s^{-1}. After cultured for 0, 2, 4, 6, 8, 10, 12, 14, 16 and 18 days, growth of the mutant and wild type were estimated by measuring the dry cell weight (DCW). A 10-mL sample was filtered through pre-weighed 5 μm microporous filter paper, and washed twice with 10 mL distilled water to remove adhering inorganic salts. The filter paper was oven-dried overnight at 105 °C. The difference between the final weight and the weight of the paper before filtration was taken as the DCW (Anandarajah et al., 2012).

The maximum growth rate was calculated by the logistic model (Yang et al., 2006):

$$B_t = B_f / \{1 + [(B_f - B_0)/B_0] \times \exp^{-4\mu_{max} t/B_f}\} \quad (1)$$

where B_f and B_0 are the dry cell weight at stationary phased and zero, respectively, t and B_t are time and the corresponding dry cell weight, and μ_{max} is the maximum growth rate, which can thus be obtained after nonlinear-fitting with Eq. (1) using the software Origin 7.0.

2.4. Chlorophyll a and carotenoids measurement

A hot methanol extraction method was used to isolate chlorophyll (Henriques et al., 2007). The concentration of chlorophyll a and total carotenoids from different samples were measured using a Spectrophotometer (UV2300, Tianmei Scientific instrument Co., Ltd., Shanghai, China).

The equations proposed by Henriques et al. (2007) were used in the analysis:

$$C_a\ (\mu g/mg) = [13.43\ A_{665} v/(lV)]/D$$

where C_a is the concentration of chlorophyll a. A_{665} means the absorbance at 665 nm. v means the volume of solvent used (mL), l is the spectrophotometric cell length (cm) and V is the sample volume (mL). D is the dry cell weight of 1 mL algal suspension.

$$C_{\text{total carotenoids}}\ (\mu g/mg) = [(1000\ A_{470} - 44.76\ A_{666}/221) v/(lV)]/D$$

where $C_{\text{total carotenoids}}$ is the concentration of total carotenoids. A_{470} means the absorbance at 470 nm. A_{666} means the absorbance at 666 nm. v means the volume of solvent used (mL), l is the spectrophotometric cell length (cm) and V is the sample volume (mL). D is the dry cell weight of 1 mL algal suspension.

2.5. Measurement of photosynthetic oxygen evolution rate

Photosynthetic oxygen evolution rate in early exponential growth phase of the HP-1 mutant and wild type were determined using a Chlorolab I (Hansatech Instruments Ltd., Norfolk, UK) equipped with a Clark-type oxygen electrode unit (Pokora and Tukaj, 2010). A sample (1 mL) withdrawn from the culture vessel was immediately placed in the measurement chamber with an outer jacket for thermostatted water (25 °C). The chamber was closed with a plunger, so that oxygen accumulated in the sample during photosynthesis. Prior to the measurement, the sample was adapted for 3 min, during which the current flowing between the anode and the cathode reached a stable level. An increase in the current was noted after the next 3 min. The cell suspension was stirred continuously during the measurement. The measurements were taken at 25 °C and a list of irradiance at 20, 40, 60, 80, 100, 200, 400, 800 and 1000 μmol photons m^{-2} s^{-1} was provided by a halogen light source (OSRAM, 150 W). Photosynthetic intensity calculated from the increase of oxygen concentration in the sample during the measurement was expressed as μmol O_2 × mg chl a^{-1} × h^{-1}.

2.6. In vivo monitoring of chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were determined in vivo using an imaging pulseamplitude-modulated fluorometer (Imaging PAM; Heinz Walz, Effeltrich, Germany). The parameters including F_v/F_m (potential maximum quantum efficiency), NPQ (nonphotochemical quenching) and Φ_{PSII} (quantum yield of PSII) were calculated, respectively, after 15 min of dark adaptation (Maxwell and Johnson, 2000).

2.7. Induction of intracellular lipid accumulation

Once all cultures had reached early stationary growth phase (after 10-day cultivation), microalgae cells were harvested by centrifugation, followed by removal of the residual nitrogen by washing with sea water. The harvested cells were inoculated with the same concentration in a 400 mL bubble column bioreactor without nitrogen. Aeration enriched with 2% CO_2 was provided at 80 mL/min. The microalgae were cultured at 25 °C under continuous illumination at 300 μmol photons m^{-2} s^{-1}. In this second stage of cultivation, lipid accumulation was triggered by the absence of a nitrogen source (Su et al., 2011). After cultured for 0, 3, 5, 7 and 9 days, the dry cell weight was measured as described before. Triplicate experiments under each culture condition were carried out.

2.8. Determination of lipid content

Microalgae cells were harvested after 0, 3, 5, 7 and 9 days in the second stage of cultivation by centrifugation at 4722*g* for 10 min. Cell pellets were lyophilized using a freeze drier (Alpha1-2LD Plus; Martin Christ GmBH, Osterode, Germany). The total lipids contained in the algal cells were extracted with a modified chloroform–methanol–water solvent system (Bligh and Dyer, 1959). After drying and grinding, approximately 50 mg dry algal powder was mixed with 5 mL methanol and 2.5 mL chloroform, and then shaken at 200 rpm for 12 h at 37 °C. The mixture was then centrifuged at 3790*g* for 10 min. The supernatant was collected and residual biomass was extracted once more. The supernatants were combined, and 5 mL chloroform and 5 mL 1% sodium chloride solution were added. After mixing and centrifuge at 3790*g* for 10 min, then the chloroform layer was removed, transferred into a vial and dried for 30 min under nitrogen flow at 60 °C. The vial was dried under vacuum (Lantian DZF-6050, Hangzhou, China) for 3 h at 60 °C, and then weighed. The total lipid content was calculated as a percentage of the dry weight of the algae.

2.9. Lipid composition and fatty acid analysis

Lipid components were analyzed using a thin-layer chromatography (TLC) system (TLC–FID, MK-6, Iatron Laboratories, Inc., Tokyo, Japan) (Fedosov et al., 2011; Chen et al., 2012). Samples were dissolved in chloroform to a concentration of 10 mg/mL, and 2 μL of solutions containing lipids was spotted onto Chromarod S-III silica coated quartz rods held in a frame. The rods were developed in a solvent system of benzene:chloroform:acetic acid (150:60:2, v/v/v) for the first migration to 7.5 cm, followed by a solution of benzene:hexane (50:50, v/v) for the second migration to 10 cm. The rods were dried at 70 °C for 3 min before they were scanned in the Iatroscan analyzer, which was operated at a flow rate of 0.16 L/min for hydrogen and 2 L/min for air. The scan speed was 30 s per rod. The recorded profiles were analyzed by SIC-480 II program. The individual lipid components were identified by co-chromatography with pure standards (sterol ester, SE; fatty acid methyl ester, FAME; triacylglycerol, TAG; diacylglycerol, DAG; phospholipids, PL; purchased from Sigma, St. Louis, MO, USA). The quantities of individual components were estimated from the peak areas of pure standards (Chen et al., 2012).

Methyl esters were generated from the microalgae lipids by heating them in a 2% H_2SO_4–methanol solution at 85 °C for 2.5 h (Yuan et al., 2011). Fatty acid methyl esters (FAMEs) were analyzed by GC using a Varian (Walnut Creek, CA, USA) 450 GC equipped with an FID detector and a Varian capillary column CP-Wax 58 (FFAP) CB (25 m × 0.25 mm × 0.20 μm). Carrier gas was nitrogen at 1 mL/min and the split ratio was 1:30. The oven temperature was initially held at 100 °C for 2 min, followed by an increase to 250 °C at 10 °C/min, which was then held for 8 min. The detection system was equipped with a flame ionization detector (FID) operating at 280 °C. FAMEs peak identification was carried out by GC–MS (NIST, 2.0) operating in the same conditions as the GC–FID. The relative percentage of the fatty acid was calculated on the basis of the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

2.10. Statistical analysis

Data were presented as the mean ± standard deviation of the mean of triplicate samples. Significant differences between means were tested using one-way analysis of variance followed by least significant difference tests, using the SPSS statistical package (version 13.0; SPSS Inc., Chicago, IL, USA) at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Heavy-ion irradiation mutagenesis

Although heavy-ion irradiation mutagenesis has been widely used in plant and microorganism breeding (Kazama et al., 2011; Wang et al., 2009), it has not been reported in microalgae breeding. In this study, heavy-ion irradiation mutagenesis was applied to *N. oceanica* IMET1, a promising species for microalgal biodiesel production. Due to the lack of referenced mutagenesis conditions, a series of heavy-ion irradiation doses was applied according to the referenced reports in plants and microorganisms (Kazama et al., 2011; Wang et al., 2009). The results revealed that the mortality rate was enhanced from approximately 15% to 89% with increasing irradiation doses (Fig. 1). According to the experience in mutagenesis breeding, high mutation frequency is usually found at high mortality rate (Wang et al., 2009). Thus, approximately 3000 clones with at least 50% mortality rate, which were irradiated

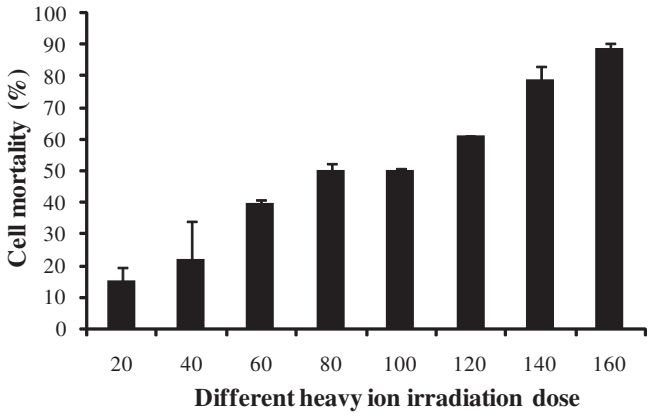

Fig. 1. Mortality of *Nannochloropsis oceanica* IMET1 treated with different dose of heavy-ion irradiation. Data are means of three repeated experiments and error bars indicate standard deviations.

under dose of 80, 100, 120, 140 and 160 Gy, respectively, were selected for further mutation screening. After large-scale screening, one mutant (HP-1) was selected with higher biomass accumulation rate. In addition, the HP-1 mutant was obtained from the highest heavy-ion irradiation dose (160 Gy) with the highest cell mortality (89%). Similar phenomenon had also been reported in EMS mutagenesis which got the desired mutant from the highest cell mortality condition (Anandarajah et al., 2012; Doan and Obbard, 2012). Consequently, the above results showed that heavy-ion irradiation could be applied as a useful candidate tool in microalgae breeding.

Generally, the induced mutagenesis, which does not require a wealth of biochemical and genetic information on the organism, presents a substantial advantage compared to genetic engineering in microalgae breeding. Previous studies showed that several physical methods were successfully applied in microalgae breeding, such as UV, γ- and X-rays (Doan and Obbard, 2012). Compared with other physical methods, such as X- and γ-rays which linear energy transfer values (LET; the energy transferred per unit length, keV μm^{-1}) were 0.2 and 2.0 keV μm^{-1}, respectively (Kazama et al., 2011), the LET value of the heavy-ion beam used in this study was extremely high, reaching 31 keV μm^{-1}. Besides, it is well known that high-LET radiation shows stronger biological effects than low-LET radiation (Zhou et al., 2006). Therefore, the heavy-ion irradiation could be regarded as a better candidate method than other physical methods in microalgae breeding.

3.2. Growth, chlorophyll and photosynthesis characteristics of the HP-1 mutant

To further characterize the higher biomass accumulation rate of the HP-1 mutant, the detailed description of the growth curve, chlorophyll content and photosynthesis characteristics for the wild type and HP-1 mutant were investigated in this study. Biomass production is one of the most important factors during the microalgal commercialization (Williams and Laurens, 2010). As showed in Fig. 2a, the biomass accumulation of the HP-1 mutant was increased by 19% at the end of the 18-day cultivation compared to wild type. The maximum growth rate of the HP-1 mutant was increased by 6% compared to that of the wild type, reaching 0.55 and 0.52 g $L^{-1}d^{-1}$, respectively. These results showed that the HP-1 mutant could be considered as a promising candidate for future microalgal commercialization.

Photosynthetic pigments are major composition of light-harvesting complex which capture light energy in microalgae photosynthesis (Williams and Laurens, 2010). Our results showed that the chlorophyll a concentration of two strains were increased after inoculation, reaching the highest level on day 4, and then decreasing in the following cultivation days (Fig. 2b). The chlorophyll a content of the HP-1 mutant was 45% higher than that of the wild type on day 4 (Fig. 2b). Similar tendency happened in the carotenoids content of two strains (Fig. 2c). A possible explanation therefore is that increased photosynthetic pigment content may enhance the ability of light energy capture, and then result in the improvement of the photosynthesis activity, thus leading to the increase of biomass accumulation in the HP-1 mutant. Similar phenomenon that increased the content of photosynthetic pigments could enhance biomass production had also been reported in several other algae strains (Hu et al., 1998; Anandarajah et al., 2012).

To further investigate the reason caused higher biomass accumulation, photosynthetic parameters, including F_v/F_m, NPQ and photosynthetic O_2 evolution, were measured in the HP-1 mutant and wild type (Fig. 3). Energy taken up by light absorption of the microalgae pigments is transformed in the process of the photosynthetic quantum conversion into photosynthesis, chlorophyll fluorescence and thermal dissipation (Maxwell and Johnson, 2000). F_v/F_m, indicating the potential maximum quantum efficiency, can directly reflect the photosynthesis activity of PS II (Li et al., 2010b), whereas NPQ is a protection mechanism to thermally dissipate excess light energy that could not be utilized for photosynthesis (Muller et al., 2001). As indicated in Fig. 3, F_v/F_m of two strains was maintained in the 10-day cultivation, and then slowly decreased in the following days, while the NPQ of the HP-1 mutant was lower than that of the wild type in early exponential growth

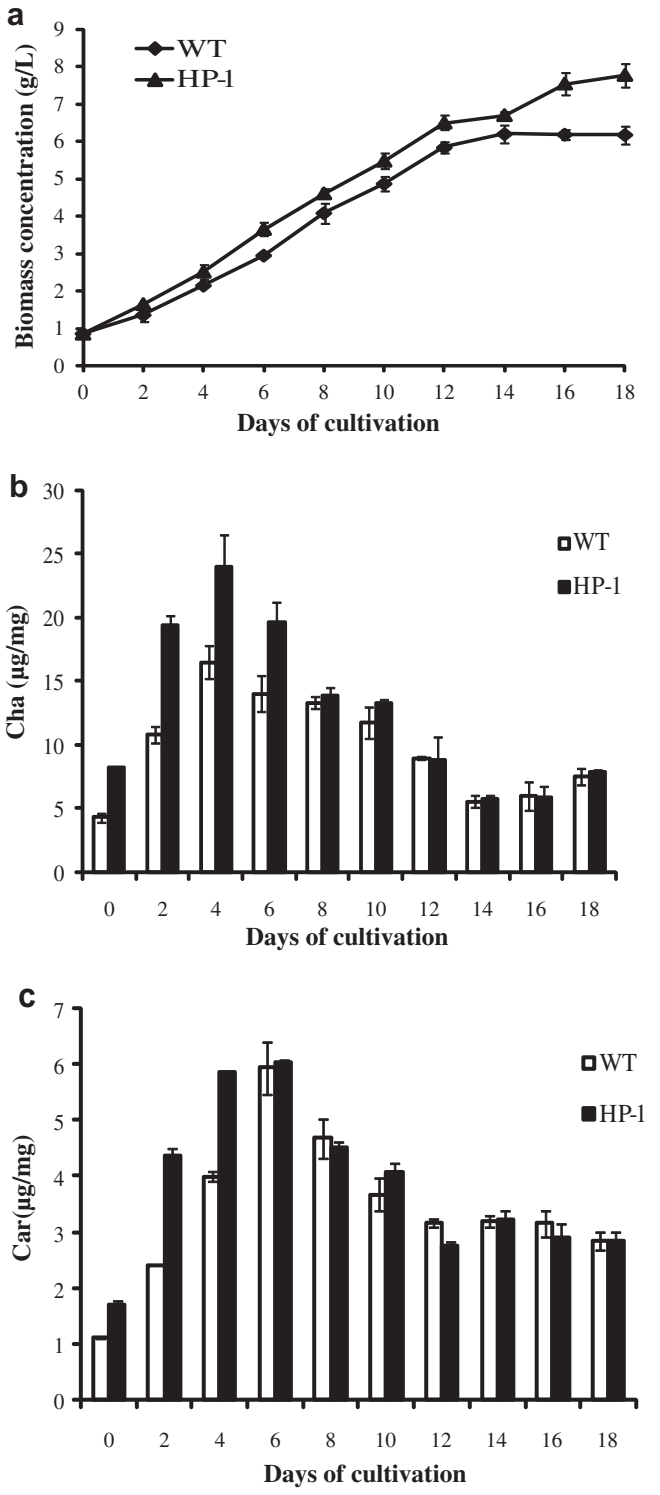

Fig. 2. Growth curve (a), chlorophyll a (Cha) (b) and carotenoids (Car) (c) content of *Nannochloropsis oceanica* IMET1 wild type (WT) and HP-1 mutant. Data are means of three repeated experiments and error bars indicate standard deviations.

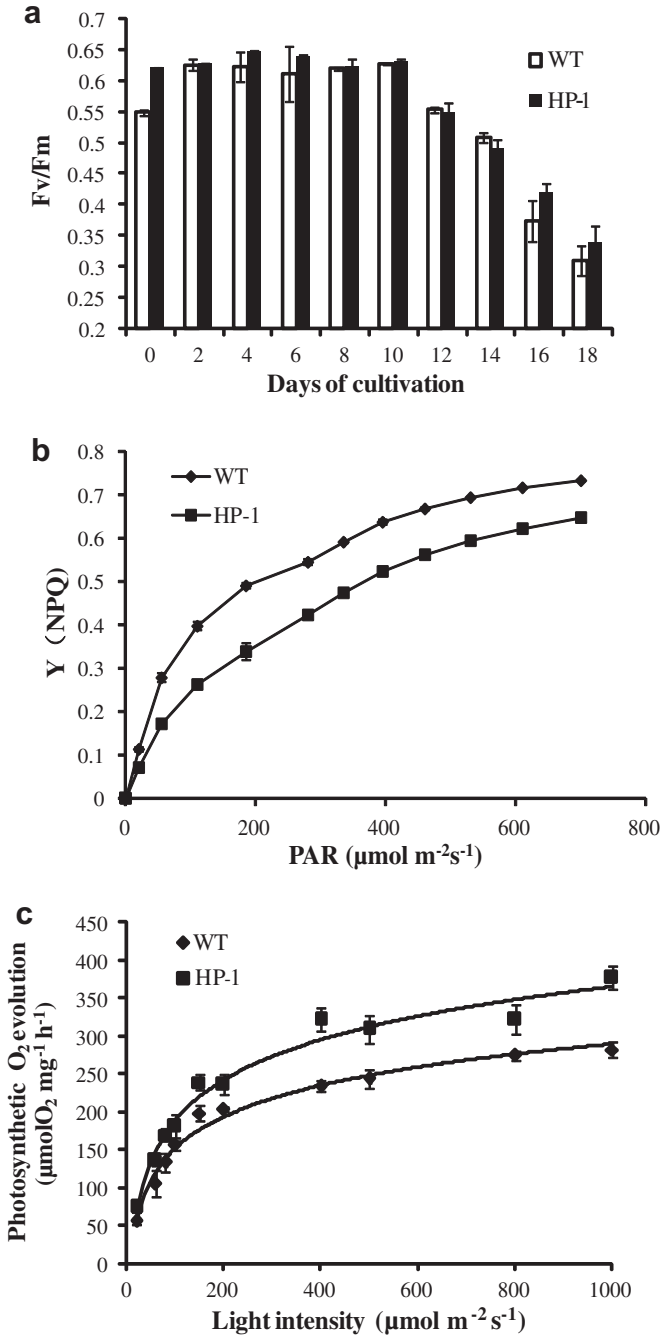

Fig. 3. Chlorophyll fluorescence parameters and photosynthetic oxygen evolution rate of *Nannochloropsis oceanica* IMET1 wild type (WT) and HP-1 mutant. (a) F_v/F_m, (b) NPQ, (c) photosynthetic oxygen evolution rate (b and c in early exponential growth phase after 4 days cultivation). PAR, photosynthetically active radiation. Data are means of three repeated experiments and error bars indicate standard deviations.

phase. Furthermore, the results of photosynthetic O_2 evolution in the HP-1 mutant was higher than that of the wild type (Fig. 3c), which implying the HP-1 mutant had higher photosynthetic rate than the wild type. The above results indicated that F_v/F_m in the HP-1 mutant was not increased compared to the wild type in early exponential growth phase, however, due to the NPQ decrease, energy flow to photosynthesis might increase in the HP-1 mutant, which was further verified with higher photosynthetic rate in the HP-1 mutant compared to the wild type. Taken together, these results suggest that the HP-1 mutant has higher photosynthetic efficiency than that of the wild type.

Microalgae biomass production is directly proportional to the efficiency with which the algal cells assimilate carbon from the atmosphere through photosynthesis (Williams and Laurens, 2010). Consequently, photosynthesis efficiency is the deciding factor in microalgae biomass production. In this study, the HP-1

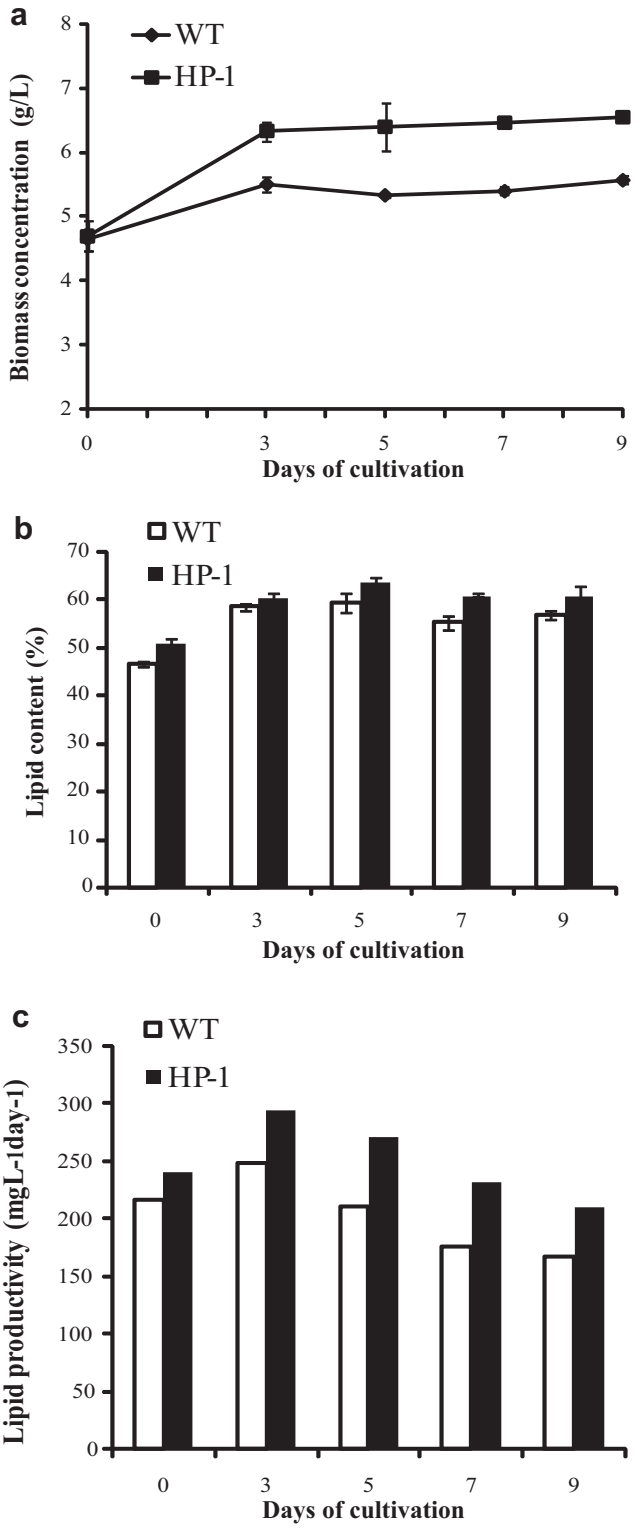

Fig. 4. Growth curve (a), lipid content (b) and lipid productivity (c) of *Nannochloropsis oceanica* IMET1 wild type (WT) and HP-1 mutant during lipid accumulation phase. Data are means of three repeated experiments and error bars indicate standard deviations.

Table 1
The lipid productivity for *Nannochloropsis oceanica* IMET1 HP-1 and other microalgae species reported in the literature.

Microalgae species	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)	Reference
Chlorella vulgaris	40	Lv et al. (2010)
Neochloris oleoabundans	133	Li et al. (2008)
Scenedesmus sp.	39	Yoo et al. (2010)
Botryococcus braunii	21	Yoo et al. (2010)
Nannochloropsis sp.	204	Rodolfi et al. (2009)
Nannochloropsis oceanica IMET1 HP-1	271	This study

mutant had significant advantage over the wild type in biomass production, which might result from its higher photosynthetic efficiency. Further investigation on the modified genes in the HP-1 mutant will deepen our knowledge in microalgae photosynthesis. It must be a difficult challenge, not only because the heavy-ion irradiation mutation are randomly spread but also because photosynthesis mechanism are very intricate. However, we believe that these results are a starting point for further genomic investigation and will be useful for the research of photosynthesis mechanism in microalgae.

3.3. Lipid accumulation characteristics of the HP-1 mutant

To further investigate the lipid accumulation of the HP-1 mutant, a second-stage cultivation model was applied. Owing to separate growth and lipid production phases, the second-stage cultivation has been proved to improve overall lipid productivity in microalgae (Su et al., 2011). In this study, after 10-day cultivation in nitrogen-repletion conditions, the two strains were harvested and inoculated into the second-stage nitrogen-deficient culture broth under an irradiance of 300 μmol photons $m^{-2}\ s^{-1}$ with the same concentration. As shown in Fig. 4a, the biomass accumulation of the HP-1 mutant was higher than that of the wild type during cultivation. The total lipid content of the HP-1 mutant was slightly higher than that of the wild type without significant difference (Fig. 4b). However, due to the significant increase of biomass accumulation, the lipid productivity of the HP-1 mutant ($271\ mg\ L^{-1}\ d^{-1}$) was 29% higher with respect to the wild type ($211\ mg\ L^{-1}\ d^{-1}$) (Fig. 4c). It is worth notice that the highest lipid productivity ($295\ mg\ L^{-1}\ d^{-1}$) appeared on day 3 in the HP-1 mutant, whereas the lipid production reached a maximum value of $4.07\ g\ L^{-1}$ on day 5 when the lipid productivity was $271\ mg\ L^{-1}\ d^{-1}$. Therefore, the lipid productivity on day 5 was selected for further comparison with previously reported microalgae species. As indicated in Table 1, the lipid productivity of the HP-1 mutant was higher than that of other reported microalgae species. It was also comparable to reported *Nannochloropsis* mutant by EMS mutagenesis ($294\ mg\ L^{-1}\ d^{-1}$) (Anandarajah et al., 2012).

To further investigate the reason caused the higher biomass productivity during lipid accumulation, the photosynthetic parameters including F_v/F_m, NPQ and Φ_{PSII} were determined. As shown in Fig. 5a, the F_v/F_m of the HP-1 mutant was higher than that of the wild type during lipid accumulation. On day 5 with the highest lipid production, the NPQ of the HP-1 mutant was lower than that of the wild type (Fig. 5b). As for the quantum yields (Φ_{PSII}), reflecting the proportion of the absorbed quanta that are converted into chemically fixed energy by the photochemical charge separation at PSII reaction centers (Baker and Oxoborough, 2004; Maxwell and Johnson, 2000), the HP-1 mutant showed higher activity than wild type (Fig. 5c). The above results showed that the photosynthetic efficiency of the HP-1 mutant was higher than that of the wild type during lipid accumulation phase, which might result in its higher biomass productivity.

Lipid productivity is one of key factors in microalgal biodiesel commercialization (Chisti, 2007). Increase in lipid productivity depends on improvements in biomass productivity and lipid content. In this study, the increase of the lipid productivity in the HP-1 mutant was mainly depended on its higher biomass productivity. Similar results had been reported in other *Nannochloropsis* mutant by

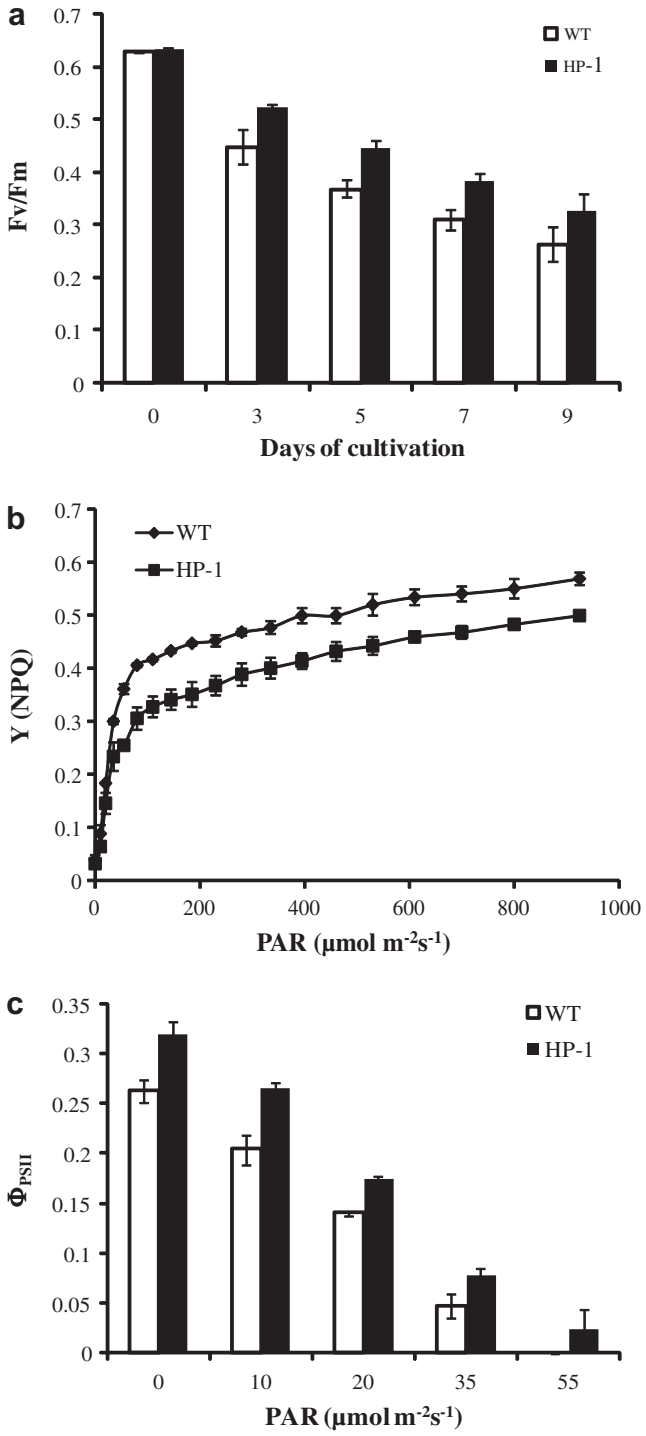

Fig. 5. Chlorophyll fluorescence parameters of *Nannochloropsis oceanica* IMET1 wild type (WT) and HP-1 mutant during lipid accumulation phase. (a) F_v/F_m, (b) NPQ, (c), Φ_{PSII} (b and c at induction of lipid accumulation for 5 days). PAR, photosynthetically active radiation. Data are means of three repeated experiments and error bars indicate standard deviations.

Table 2
Lipid composition analyses of *Nannochloropsis oceanica* IMET1 wild type (WT) and HP-1 mutant in initial inoculation and induction of lipid accumulation for 5 days.

Lipid composition	0 days		5 days	
	WT	HP-1	WT	HP-1
SE	4.5 ± 0.1	6.8 ± 0.8	4.4 ± 1.0	3.0 ± 0.6
FAME	0.9 ± 0.1	1.1 ± 0.1	1.3 ± 0.2	1.4 ± 0.1
TAG	28.2 ± 1.5	39.6 ± 2.0*	49.4 ± 1.4	56.5 ± 1.8*
FS&DAG	2.3 ± 0.2	2.5 ± 0.4	5.6 ± 0.2	5.7 ± 0.6
PL	64.1 ± 2.5	50.0 ± 1.6*	39.3 ± 1.6	33.4 ± 1.6*

Data are given as means ± S.D., $n = 3$.
* Difference is statistically significant with the wild type ($p < 0.05$).

Table 3
Fatty acid profiles analyses of *Nannochloropsis oceanica* IMET1 wild type (WT) and HP-1 mutant in initial inoculation and induction of lipid accumulation for 5 days.

Fatty acids	0 days		5 days	
	WT	HP-1	WT	HP-1
14:0	8.3 ± 0.1	7.9 ± 0.5	7.9 ± 0.2	8.7 ± 0.4
16:0	34.9 ± 0.3	31.4 ± 1.2	45.1 ± 1.3	47.0 ± 0.8
16:1	25.0 ± 0.6	26.0 ± 0.2	23.4 ± 0.9	21.5 ± 1.0
18:0	3.6 ± 0.2	3.2 ± 0.1	2.4 ± 0.2	2.4 ± 0.5
18:1	13.5 ± 0.9	12.7 ± 0.7	8.4 ± 1.0	8.2 ± 0.4
18:2	2.00 ± 0.2	2.5 ± 0.1	2.6 ± 0.1	2.4 ± 0.3
20:4	3.4 ± 0.3	4.4 ± 0.3	3.7 ± 0.2	3.8 ± 0.6
20:5	9.4 ± 0.9	11.8 ± 1.3	6.8 ± 0.3	6.1 ± 0.4
SFA[a]	46.8 ± 0.6	42.5 ± 1.8	55.4 ± 1.7	58.1 ± 1.7
MUFA[b]	38.5 ± 1.4	38.7 ± 1.0	31.8 ± 1.9	29.7 ± 1.5
PUFA[c]	14.8 ± 1.4	18.7 ± 1.8	12.8 ± 0.6	12.2 ± 1.3
UFA[d]	53.2 ± 2.8	57.5 ± 2.7	44.6 ± 2.5	42.0 ± 2.8

Data are given as means ± S.D., $n = 3$.
[a] SFA: percentage of saturated fatty acids (% of total fatty acids).
[b] MUFA: percentage of monounsaturated fatty acids (% of total fatty acids).
[c] PUFA: percentage of polyunsaturated fatty acids (% of total fatty acids).
[d] UFA: percentage of unsaturated fatty acids (% of total fatty acids).

EMS mutagenesis (Anandarajah et al., 2012). Further, we found the reason caused the higher biomass productivity lied in its higher photosynthesis efficiency. Taken together, we suggest increased photosynthesis efficiency of the HP-1 mutant result in its higher lipid productivity than that of the wild type.

3.4. Lipid composition and fatty acid profile analyses of the HP-1 mutant

To further analyze the lipid characteristics for biodiesel production, the lipid composition and fatty acid profiles of the HP-1 mutant and wild type were determined (Tables 2 and 3). In the initial inoculation, the lipid composition analysis showed the polar lipid was the main composition in the total lipid of the HP-1 mutant reaching 50.0%, in comparison to 64.1% of the wild type, while triacylglycerol (TAG) content reaching 39.6% in HP-1 mutant and 28.2% in wild type, respectively. Other component contents, including sterol esters (SE), fatty acid methyl ester (FAME), free sterol (FS) and diacylglycerol (DAG), were lower than both TAG and polar lipid contents in two strains. After induction of lipid accumulation for 5 days in nitrogen-deficient culture broth, the TAG content was obviously increased, while the polar lipid content was remarkably decreased in two strains (Table 2). Further analysis indicated that the TAG content of the HP-1 mutant was increased by 14% compared to the wild type, whereas the polar lipid content was decreased by 15%. TAG is the best substrate to produce biodiesel (Xu et al., 2006; Chisti, 2007; Hu et al., 2008; Rodolfi et al., 2009), while polar lipid is not popular as it can result in loss of biodiesel production due to precipitation and saponification (Balasubramanian and Obbard, 2011; Chen et al., 2012). In this study, the HP-1 mutant showed the characteristics of higher TAG and lower polar lipid level than that of the wild type, indicating that the HP-1 mutant could be considered as an ideal candidate for biodiesel production.

Fatty acid composition of the HP-1 mutant and wild type in initial inoculation and induction of lipid accumulation for 5 days were analyzed. Fatty acids with less than 1% compositions were not included in Table 3. The results showed that C14:0 (myristic acid), C16:0 (palmitic acid), C18:1 (oleic acid) and C20:5 (eicosapentaenoic acid) were major fatty acids of two strains. After induction of lipid accumulation for 5 days, the saturated fatty acids were increased, accompanying with unsaturated fatty acids deceased. No significant difference between HP-1 mutant and wild type were found for all fatty acid measured in the experiment. Although the TAG content of the HP-1 mutant was higher than that of the wild type, its fatty acid composition was not significantly changed, suggesting that the changed fatty acid composition due to higher TAG level may be eliminated by the variation from lower polar lipid. Notably, we found that significantly variation existed in fatty acid composition of different *Nannochloropsis* (Li et al., 2010a; Doan et al., 2011). Generally, higher lipid productivity was accompanied by lower percentage of polyunsaturated fatty acids in *Nannochloropsis* (Li et al., 2010a). In this study, our results were consistent with the previous report.

4. Conclusion

Microalgal biodiesel has not been widely commercialized due to its high cost. Strain improvement has been considered to have the greatest impact on the decrease in the cost of microalgal biodiesel production. In this study, one mutant (HP-1) was obtained from *Nannochloropsis* by heavy-ion irradiation mutagenesis. Compared to the wild type, its lipid productivity and TAG content was increased by 29% and 14%, respectively, while polar lipid was decreased by 15%, indicating that it could be considered as a valuable candidate for microalgal biodiesel production. Much work remains to be done in strain improvement to make microalgal biodiesel commercialization.

Acknowledgements

This study was carried out in the framework of QIBEBT-BOEING Joint Research Laboratory for Sustainable Aviation Biofuels Collaboration Agreement, and also supported by Knowledge Innovation Program of the Chinese Academy of Sciences (No. KSCX2-EW-Q-22), Natural Science Foundation of Shandong Province (No. ZR2011DQ007) and CAS/SAFEA International Partnership Program for Creative Research Teams.

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