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RESEARCH ARTICLE

Effects of laser irradiation on a bloom forming cyanobacterium *Microcystis aeruginosa*

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Abstract Effects of laser irradiation on photosystem II (PS II) photochemical efficiencies, growth, and other physiological responses of *Microcystis aeruginosa* were investigated in this study. Results indicate that laser irradiation (wavelengths 405, 450, 532, and 650 nm) could effectively inhibit maximal PS II quantum yield (Fv/Fm) and maximal electron transport rates (ETR_{max}) of *M. aeruginosa*, while saturating light levels (E_k) of *M. aeruginosa* did not change significantly. Among the four tested wavelengths, 650 nm laser (red light) showed the highest inhibitory efficiency. Following 650 nm laser irradiation, the growth of *M. aeruginosa* was significantly suppressed, and contents of cellular photosynthetic pigments (chlorophyll a, carotenoid, phycocyanin, and allophycocyanin) decreased as irradiation dose increased. Meanwhile, laser irradiation enhanced the enzyme activities of superoxide dismutase (SOD) and peroxidase (POD) in *M. aeruginosa* cells. Lower irradiation doses did not change the intracellular microcystin contents, but higher dose irradiation (>1284 J cm^{-2}) caused the release of microcystin into the culture medium. Transmission electron microscope examination showed that the ultrastructure of *M. aeruginosa* cells was destructed progressively following laser irradiation. Effects of laser irradiation on *M. aeruginosa* may be a combination of photochemical, electromagnetic, and thermal effects.

Keywords Laser irradiation · *Microcystis aeruginosa* · Growth inhibition · Photosynthetic activity · Antioxidant enzyme · Microcystin

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Introduction

Overloading of nutrients leads to the frequent occurrence of algal blooms in lakes and reservoirs worldwide (Dodds et al. 2009; Paerl et al. 2011), which reduces water transparency, deteriorates water quality, affects other aquatic organisms, and decreases biodiversity (Hudnell 2010). The wide spread of algal blooms limits the use of lakes and reservoirs for drinking water sources and recreational activities (Weirich and Miller 2014). Toxins produced by cyanobacteria can pose serious risks to aquatic organisms, even human beings, and may cause fatal consequences (Carvalho et al. 2013; Steffen et al. 2014). Therefore, the prevention and control of algal blooms are of great importance for the health of humans and animals.

Controlling methods of algal blooms are usually divided into physical, chemical, and biological ones. Physical methods such as salvage, filtration, and flotation are commonly used when algal bloom occurs. Sonication is a more recently developed approach (Wu et al. 2012), and other physical methods such as ultraviolet radiation (Holzinger and Lutz 2006) and gamma-ray irradiation (Zheng et al. 2012) have also been considered as feasible methods to control algal blooms. But they are not widely applied probably because of their high cost and potential risks to other aquatic organisms. Chemical methods are economic and efficient in controlling algal blooms. Chemicals such as ozone (Li et al. 2011), chlorine dioxide (Zhou et al. 2014a), hydrogen peroxide (Matthijs

et al. 2012), potassium ferrate (Zhou et al. 2014b), and copper sulfate (Song et al. 2011) were previously studied. Flocculation using clays, soils, and sediments modified by chitosan was reported to be an effective way to remove harmful algae (Zou et al. 2006). Pinho et al. (2015) reported that a solar-driven TiO_2 photocatalytic process can efficiently destroy the cyanobacteria *Microcystis aeruginosa*. However, chemical methods have been applied less frequently recently, primarily due to the environmental safety concerns (Jia et al. 2013). Biological methods using macrophytes, fish, zooplankton, and filter-feeding bivalves were also successfully applied in controlling the growth of algae (He et al. 2014; Hong et al. 2008; Lu et al. 2006; Wang et al. 2012). Most biological methods have a good effect on algae control in small scales. However, applying these methods in large natural water bodies can be difficult and may have limited effectiveness due to the complicated ecological processes involved and a considerably long time to take effect.

Laser is a single-color light with very high energy density and directivity. Presently, laser has been widely used in various fields such as welding, cutting, lighting, communication, phototypesetting, and breeding. Biostimulation by low-intensity laser irradiation on agricultural seeds has been studied previously. Hernández et al. (2015) reported that laser at a 650 nm wavelength induced temperature changes in maize seeds, and the thermal effect of laser was believed to associate with biostimulation. Laser irradiation was found to have considerable biological effects on the metabolism of wheat during germination and later vegetative growth (Jamil et al. 2013). Suitable doses of laser irradiation can enhance the physiological attributes such as photosynthetic rate, chlorophyll content, transpiration rate, and water use efficiency and increase production of biochemicals including proteins, carotenoids, enzyme activities, essential oil, and abscisic acid (El-Kereti et al. 2013; Gao et al. 2015; Perveen et al. 2011). Some researchers have pointed out that laser irradiation could increase the biomass of hydrophytes, accelerated heavy metal accumulation, and phytoremediation of phosphorus and nitrogen compounds from wastewater and contaminated soil (Dobrowolski et al. 2012). In the meanwhile, some researchers have reported that laser irradiation could inhibit the marine plankton growth like *diatoms* and *dinoflagellate*, preventing biofouling for marine environmental sensor (Nandakumar et al. 2003, 2009; Delauney et al. 2010). The effect of laser irradiation on organisms can be related to the exposure dosage. The possible mechanisms of biostimulation from laser irradiation include heat, light, and electromagnetic effects. Lower dose of laser irradiation usually stimulates organisms, but higher dose of laser irradiation can have inhibitory or even lethal effects. Therefore, we hypothesize that higher laser irradiation intensity may induce physiological damages to algae.

Information on the effects of high-intensity laser irradiation on growth, physiological attributes, and ultrastructural changes of phytoplankton remains unclear to date. Here, we propose that laser irradiation could be an effective way to control the growth of phytoplankton and the effectiveness of laser irradiation on a bloom-forming cyanobacterium *M. aeruginosa* was investigated. Therefore, the objectives of this study were to investigate the inhibitory effects of laser irradiation on *M. aeruginosa* and to elucidate the possible mechanisms involved in the processes.

Materials and methods

Materials

M. aeruginosa FACHB-915 was obtained from the Freshwater Algal Culture Collection of Institute of Hydrobiology, Chinese Academy of Sciences. The cell culture was conducted in 500 mL sterilized glass flasks containing 200 mL of BG11 medium, which were placed under fluorescent lamps at 2000 lx with a 12-h/12-h light dark/cycle, at 25 ± 1 °C. Four low-intensity lasers (50 mW power, beam diameter 3 mm, light intensity 0.71 W cm^{-2}) at visible wavelength (405, 450, 532, and 650 nm) and a high-power laser (1 W power, beam diameter 6 mm, light intensity 3.57 W cm^{-2}) at wavelength of 650 nm were used in the experiment. All lasers were purchased from Xi'an MingHui Optoelectronic Technology Co., Ltd, China.

Irradiation experiment

A mixed algal suspension with a density of 7.5×10^6 cells mL^{-1}, which was at the exponential growth phase, was prepared. Laser irradiation experiments were performed as illustrated in Fig. 1. Laser was held perpendicularly on an iron stand. Firstly, 5 mL of the prepared algal suspension was added into a 10 mL centrifuge tube wrapped with aluminum foil. Then, the centrifuge tube was placed 2 cm beneath the laser in a tube rack. After

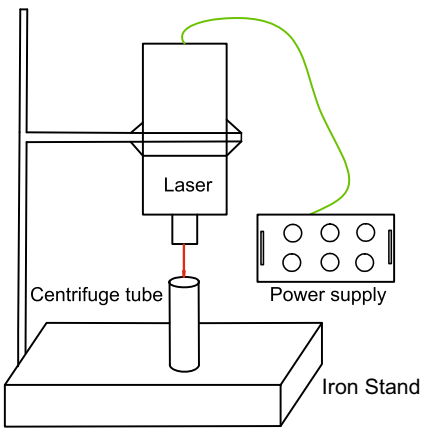

Fig. 1 Schematic diagram of laser irradiation experiment

the laser was turned on, the algal suspension was irradiated for different durations (50 mW laser irradiated for 30 min, 1 W laser irradiated for 3, 6, 9, 12, and 15 min). The irradiation dosage was calculated by multiplying light intensity by exposure time, which has a unit of Joules per square centimeter. After exposure, the algal suspension was mixed and harvested for the analysis. An aliquot of 1 mL algal mixture was prepared for cell ultrastructure imaging while 5 mL was used for the measurement of other parameters. All experiments were performed in triplicate. The low-intensity lasers (50 mW) were used to select the laser wavelength, and the high-power laser (1 W) was used subsequently to study the effects of laser irradiation on *M. aeruginosa.*

Cell growth analysis

After being irradiated by a 650 nm laser for 642, 1284, 1926, 2568, and 3210 J cm^{-2} respectively, 5 mL algal suspension of *M. aeruginosa* was inoculated in 250 mL Erlenmeyer flasks containing 100 mL of BG11 previously sterilized at 120 °C for 15 min in an autoclave. Then, the samples were cultured under fluorescent lamps at 2000 lx with a 12 h/12 h light/dark cycle, at 25 ± 1 °C. Cell density of *M. aeruginosa* was determined using a flow cytometer (Accuri C6, BD, USA). The sampling volume was 0.5 mL, and the flow cytometer was operated at a flow rate of 10 μL min^{-1}. The cell density analysis was performed every 2 or 3 days.

Fluorescence measurements

Chlorophyll fluorescence measurements of *M. aeruginosa* were conducted using a Water-PAM fluorometer (Walz, Germany). After a 30 min irradiation (50 mW lasers) at four wavelengths (dosage 1278 J cm^{-2}), 5 mL algal suspension was diluted to 50 mL and incubated in the dark for 5 min. Then, the maximum photosystem II (PS II) optical quantum yield (Fv/Fm), maximal relative electron transport rates through PS II (ETR_{max}), and saturating light levels (E_k) were determined in a cuvette containing 3 mL of algal suspension.

Photosynthetic pigment measurement

Chlorophyll *a* (Chl. *a*) and carotenoid were determined spectrophotometrically using an Agilent Cary 60 UV-Vis spectrophotometer after algal cells were irradiated by a 650 nm laser for 642, 1284, 1926, 2568, and 3210 J cm^{-2}, respectively. Firstly, samples were filtered by Whatman 1.2-μm GF/C glass fiber filters and then extracted with 5 mL of 95 % ethanol overnight at 4 °C. After that, the samples were centrifuged at 4000 rpm for 10 min and the absorbance of the supernatant was determined at 665, 649, and 470 nm. The cellular pigment contents were calculated using Eqs. (1) and (2) as described by Xiao et al. (2010):

$$\text{Chl. } a = 13.7 \times A_{665} - 5.76 \times A_{649} \quad (1)$$

$$\text{Carotenoid} = \frac{1000 \times A_{470} - 2.05 \times Chl.a}{245} \quad (2)$$

Phycocyanin (PC) and allophycocyanin (APC) were determined as described by Bennett and Bogorad (1973). Samples were resuspended in 0.1 M phosphate-buffered saline (PBS) and homogenized by an ultrasonic cell pulverizer (JY92-IIN, Xinzhi Co., China, 200 W, ultrasonic time 5 s; rest time 4 s; cycle 60) with ice-bath. Then, the homogenate was centrifuged at 10,000 rpm for 10 min, and the absorbance of the extraction was determined at 615 and 652 nm. Contents of PC and APC were calculated using Eqs. (3) and (4):

$$\text{PC} = \frac{A_{615} - 0.474 \times A_{652}}{5.34} \quad (3)$$

$$\text{APC} = \frac{A_{652} - 0.208 \times A_{615}}{5.09} \quad (4)$$

Ratios of carotenoid/Chl. *a*, PC/Chl. *a*, and APC/Chl. *a* were also calculated. Carotenoid/Chl. *a* is correlated with the capacity of light-protecting mechanisms (Kuster et al. 2004). PC/Chl. *a* and APC/Chl. *a* reflect the regulation of phycobilisome content according to light intensity and quality (Jiang and Qiu 2005).

Enzyme activity assay

After being irradiated by a 650-nm laser for 642, 1284, 1926, 2568, and 3210 J cm^{-2}, algal cells were harvested by centrifugation at 4000 rpm for 10 min at 4 °C, and then the supernatant was discarded. After that, the pellets were resuspended in 2.5 mL PBS. Then, the cells were homogenized with ice-bath using the ultrasonic cell pulverizer (200 W, ultrasonic time 3 s; rest time 5 s; cycle 60). The homogenate was centrifuged at 11,000 rpm for 15 min at 4 °C. The supernatant, cell-free enzyme extract, was used to measure the total protein contents and activities of superoxide dismutase (SOD) and peroxidase (POD). The total extracted protein was determined by binding of Coomassie Brilliant Blue G-250 to protein according to Bradford (1976), and the enzyme activities were determined using commercial kits from Nanjing Jiancheng Biology Engineering Institute. SOD and POD activities were expressed as enzyme-activity unit per milligram of protein (U mg $protein^{-1}$).

Microcystin analysis

Samples were centrifuged at 4000 rpm for 10 min after being irradiated by a 650 nm laser for 642, 1284, 1926, 2568, and 3210 J cm^{-2}. Then, the supernatant was filtrated through a

0.45 μm glass fiber filter and concentrated for extracellular (in culture medium) microcystin (MC) analysis according to Cong et al. (2006). The residue was freeze-dried, then 1 mL 75 % methanol was added into the centrifuge tubes. Thereafter, the cells were disrupted by ultrasonication (300 W, 1 min) and extracted in the dark for three times. Finally, the homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was filtered using a 0.45 μm PTFE syringe filter for intracellular MC analysis.

Concentration of MC was determined using a high-performance liquid chromatography system (Waters 2695, USA) coupled with a photodiode array detector (Waters 2996, USA) operated at 238 nm according to the method of Wiedner et al. (2003).

Cell ultrastructure imaging

The samples of the *M. aeruginosa* cells were treated by different laser irradiation doses (1284, 2568, and 3210 J cm^{-2}) and then fixed with 2.5 % glutaral and prepared by ultrathin sections. Prepared samples were observed using a HT-7700 transmission electron microscope (TEM) (Hitachi, Japan).

Temperature measurement

In order to investigate the heat effect of laser irradiation on *M. aeruginosa*, temperature of the algal suspension irradiated by 1 W 650 nm laser was measured every minute using a thermometer (KangSheng Pharmaceutical Technology Co., Ltd, China). An aliquot of 5 mL algal suspension with cell density of 7.5×10^6 cells mL^{-1} was added into a 10 mL centrifuge tube, and the irradiation experiment was conducted as described in Fig. 1.

Statistics

All data in this study were expressed as the means ± standard deviation of three replicates. Differences between treatments and control were tested using one-way analysis of variance (ANOVA), and differences between treatments were tested by least significant difference (LSD) test using PASW Statistics 18 (SPSS Inc.). The significance level was set at 0.05.

Results

Effect of laser irradiation on chlorophyll a fluorescence parameters

Changes of Fv/Fm, ETR_{max}, and E_k of *M. aeruginosa* cells treated with lasers of four wavelengths for 30 min were presented in Fig. 2. Irradiation with lasers of four different wavelengths led to a decrease in Fv/Fm values compared to the control ($p < 0.05$). ETR_{max} of *M. aeruginosa* declined significantly under laser irradiation at 405, 450, and 650 nm wavelengths ($p < 0.05$), but it did not change obviously after being treated with a laser at 532 nm ($p = 0.677$). In contrast, E_k of *M. aeruginosa* irradiated with all the lasers had no significant change compared to the control ($p > 0.057$). Results of Fv/Fm and ETR_{max} indicated that the 650 nm laser had the highest inhibitory effect on photosynthetic efficiency followed by 405, 450, and 532 nm lasers according to LSD test. Therefore, subsequent experiments were performed using the 650 nm laser.

Effect of laser irradiation on cell growth

Results of the growth inhibition experiment showed that the 650 nm laser can effectively inhibit the growth of *M. aeruginosa*, and the inhibitory effects enhanced with the increase of exposure doses (Fig. 3). The cell density in control increased from 3.6×10^5 to 1.1×10^7 cells mL^{-1} after incubation for 20 days. Cell densities in two treatments that received 642 and 1284 J cm^{-2} laser irradiation increased to 7.9×10^6 and 3.0×10^6 cells mL^{-1}, respectively, whereas cells that received 1926, 2568, and 3210 J cm^{-2} laser irradiation showed no growth within 20 days, suggesting the apoptosis of *M. aeruginosa* cells.

Effects of laser irradiation on pigment contents

Chl. *a* in solution showed no significant difference from that of control after being treated with 642 and 1284 J cm^{-2} irradiation ($p = 0.794$, $p = 0.577$) (Fig. 4a), but decreased at higher laser irradiation doses ($p < 0.01$). When irradiation dose reached 3210 J cm^{-2}, Chl. *a* decreased by 57.5 % to 1.65 mg L^{-1}. Different from the trend of Chl. *a*, the carotenoid content of *M. aeruginosa* decreased after exposure to 642 J cm^{-2} laser irradiation. After 3210 J cm^{-2} irradiation, carotenoid content decreased by 76.4 % to 0.205 mg L^{-1}. Meanwhile, PC and APC show trends similar to Chl. *a* (Fig. 4b). Their concentrations did not change significantly ($p > 0.532$) after cells were treated with 642 J cm^{-2} irradiation and then decreased at higher doses (more than 1926 J cm^{-2}, $p < 0.01$). After exposure to 3210 J cm^{-2} irradiation, PC and APC concentrations dropped to 26.1 and 13.5 % of the control, respectively. In addition, carotenoid/Chl. *a* ratio showed a significant decrease ($p < 0.01$) with increasing irradiation dosage (Fig. 4c). In contrast, the PC/Chl. *a* ratio decreased significantly ($p < 0.05$) after receiving 2568 J cm^{-2} laser irradiation, while APC/Chl. *a* shows a significant decrease ($p < 0.01$) compared to the control after receiving 1284 J cm^{-2} irradiation (Fig. 4d).

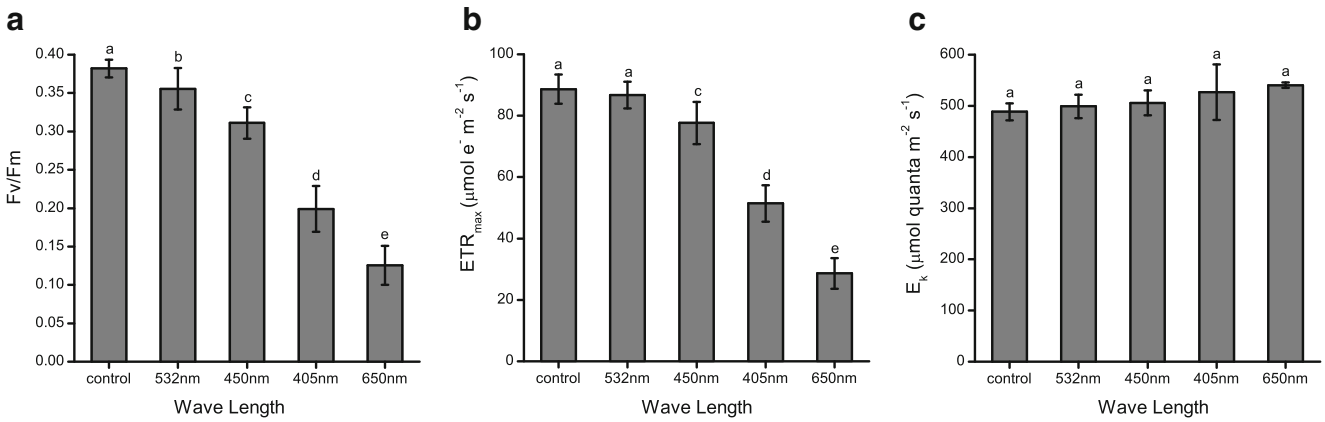

Fig. 2 Changes in Fv/Fm (**a**), ETR_{max} (**b**), and Ek (**c**) of *M. aeruginosa* (FACHB-915) irradiated by lasers with four wavelengths for 30 min (1278 J cm^{-2}). *Letters* represent whether there are differences between values of groups ($p < 0.05$)

Effects of laser irradiation on SOD and POD activities

Changes of total protein content and SOD and POD activities during exposure were presented in Fig. 5. The results showed that the protein content decreased when receiving 1926 J cm^{-2} or higher doses and decreased by 80 % compared to the control with 3210 J cm^{-2} irradiation. Both SOD and POD activities showed no significant change receiving 1284 J cm^{-2} irradiation ($p = 0.106$, $p = 0.641$), but increased significantly ($p < 0.05$) at higher exposure doses compared to the control (Fig. 5b, c). When the irradiation dose reached 3210 J cm^{-2}, the SOD and POD activities were 1.6 and 6.7 times higher than the control.

Effects of laser irradiation on MC content

Only microcystin-LR (MC-LR) was detected. Intracellular and extracellular MC-LR contents showed no significant difference from the control ($p > 0.296$) after receiving 642 and 1284 J cm^{-2} irradiation (Fig. 6). In contrast, intracellular MC started to decrease at higher doses, and a 42.9 % decrease was observed when receiving 3210 J cm^{-2} irradiation. Meanwhile, extracellular MC content increased when receiving 1926 J cm^{-2} or higher irradiation doses ($p < 0.01$). The extracellular MC was 3.5 times the control when receiving 3210 J cm^{-2} irradiation, whereas the total MC contents (intracellular and extracellular MC) showed no significant difference ($p > 0.05$) in the system.

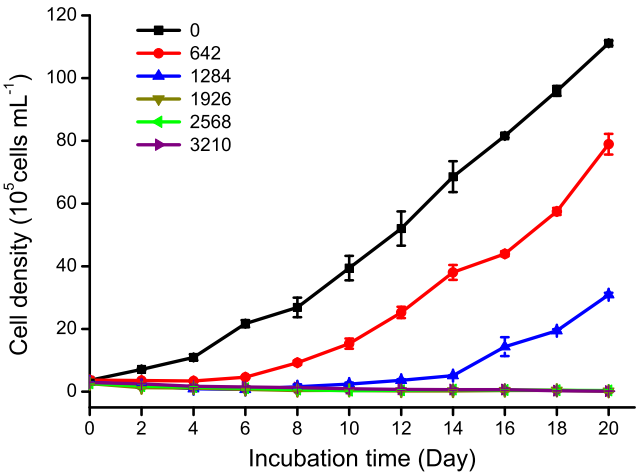

Fig. 3 Changes of *M. aeruginosa* cell density during incubation after being irradiated with different doses. *Error bar* represents standard deviation of the three replicates

Effects of laser on ultrastructure of *M. aeruginosa*

The ultrastructure was compared between control cells and those treated with 1284, 2568, and 3210 J cm^{-2} laser irradiation (Fig. 7). Control cells showed a normal *M. aeruginosa* cell ultrastructure with gelatinous layer, cell wall, cell membrane, cytoplasm, and nucleoid, and the organelles (carboxysome, thylakoid, cyanophycin granules, polyphosphate bodies, and gas vesicles) presented clearly in the nucleoplasmic area (Fig. 7a). When treated with 1284 J cm^{-2} laser irradiation, thylakoids were damaged, polyphosphate bodies and cyanophycin granules can still be observed in cells, and the nucleoid began to diffuse all around (Fig. 7b). With 2568 J cm^{-2} irradiation, cell wall deformation occurred, the shapes of thylakoids became fuzzy while the cyanophycin granules still existed, the nucleoid dispersed considerably, and larger vacuoles appeared in the nucleoplasm (Fig. 7c). With 3210 J cm^{-2} irradiation, plasmolysis was observed and thylakoid dissolved entirely, while the numbers of polyphosphate bodies, cyanophycin granules, and gas vesicles decreased drastically (Fig. 7d).

Temperature changes

Temperature of the algal suspension irradiated by red light laser increased with the increase of irradiation doses (Fig. 8). The increasing rate went up during the first 7 min and then

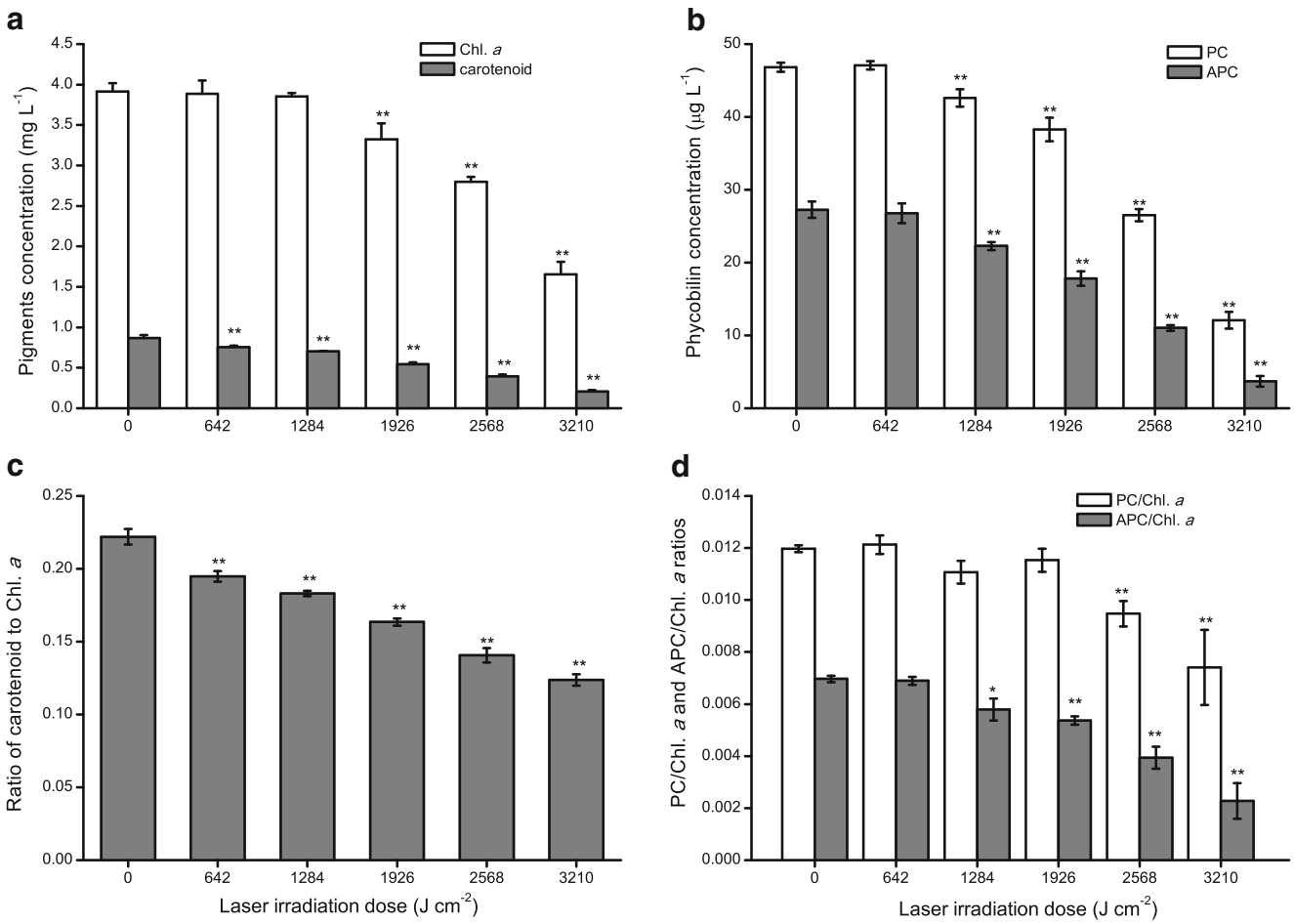

Fig. 4 Changes of Chl. *a* and carotenoid (**a**), PC and APC (**b**), ratio of carotenoid to Chl. *a* ratio (**c**), and ratios of PC and APC to Chl. *a* (**d**) in *M. aeruginosa* cell culture receiving different laser irradiation doses. *Error bar* represents standard deviation of the three replicates. *($p < 0.05$) and **($p < 0.01$)

declined in the last 8 min. Subsequently, the temperature of algal suspension irradiated by laser increased from 26.2 to 42.1 °C while the temperature of the control did not change significantly ($p > 0.05$).

Discussion

The influence of biostimulation processes on organisms is linked to laser power density and wavelength. The inhibitory

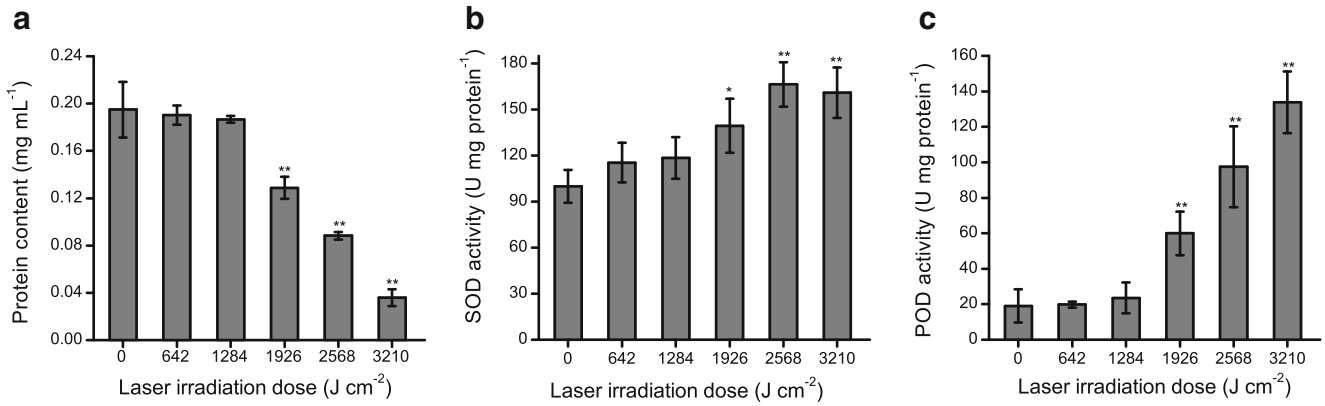

Fig. 5 Effects of laser irradiation on protein content (**a**) and the activities of SOD (**b**) and POD (**c**) of *M. aeruginosa* after receiving different laser irradiation doses. *Error bar* represents standard deviation of the three replicates. *($p < 0.05$) and **($p < 0.01$)

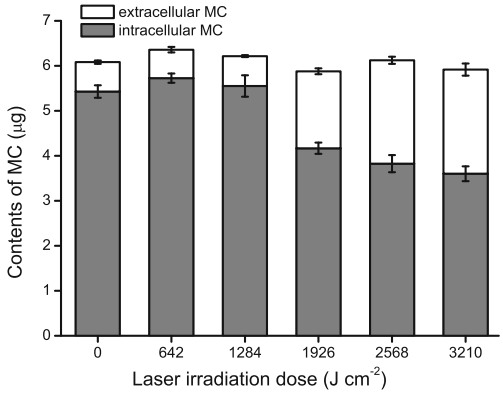

Fig. 6 Effects of laser irradiation on intracellular and extracellular MC contents of the *M. aeruginosa* culture. *Error bar* represents standard deviation of the three replicates

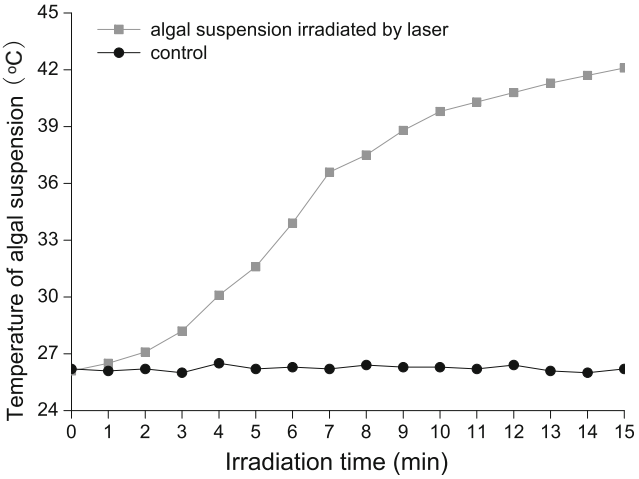

Fig. 8 Temperature changes of algal suspension treated by different durations of laser irradiation

effect of laser irradiation on *M. aeruginosa* cells was wavelength dependent, and red light (650 nm) laser had the highest inhibitory effect. This, on the one hand, may be related to a better absorption of red light by *M. aeruginosa* cells, which has a light green color. On the other hand, the phytochromes of *M. aeruginosa* are sensitive to red light and infrared radiation (Chen et al. 2005b). So red light laser could induce photodamage to *M. aeruginosa*. Dobrowolski et al. (2012) also found that plants irradiated with red light laser (660 nm, 20 mW power) showed the biggest increase in biomass production and better adaptation to unfavorable environmental conditions.

Fv/Fm is often used to evaluate PS II damage caused by light, pollutants, and other environmental stresses, and a low Fv/Fm value means lower photosynthetic efficiency (Yang et al. 2015). The decrease of Fv/Fm and ETR_{max} might indicate that an important portion of the PS II reaction center was damaged and the electron transport chain might be affected.

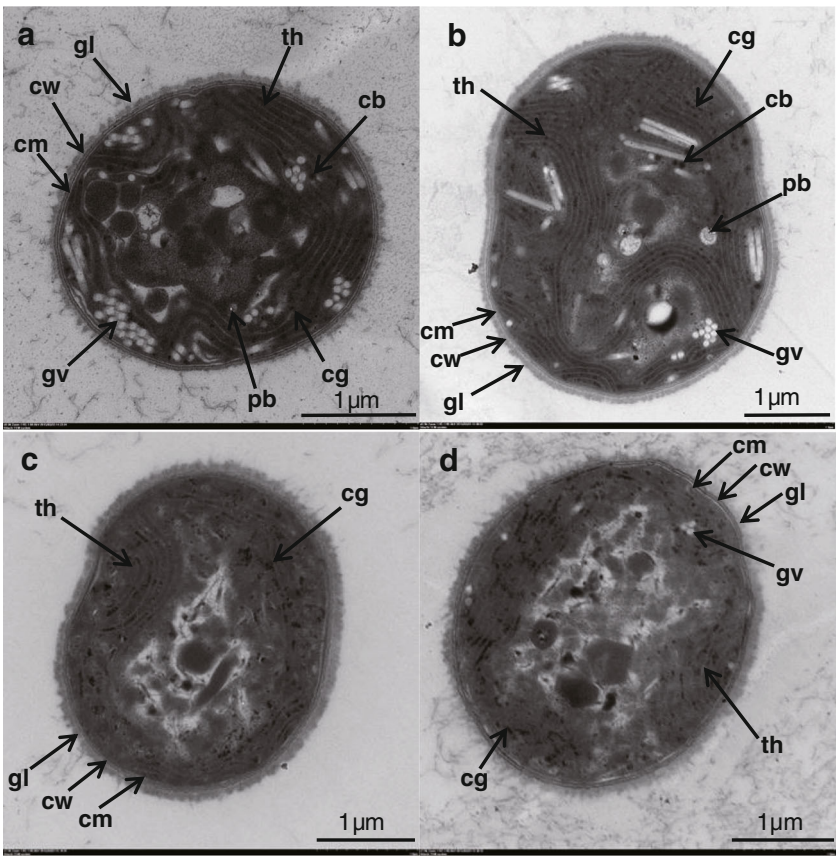

Fig. 7 Transmission electron microscopic images of *M. aeruginosa* cells receiving different laser irradiation doses: **a** control; **b** 1284 J cm^{-2}; **c** 2568 J cm^{-2}; **d** 3210 J cm^{-2}. *gl* gelatinous layer; *cw* cell wall; *cm* cell membrane; *th* thylakoid; *cg* cyanophycin granules; *cb* carboxysome; *pb* polyphosphate bodies; *gv* gas vesicles

E_k is used as an index of the photo-acclimation state of phytoplankton. High E_k values are related to growth at high irradiance and indicate a relatively greater capacity for light-saturated photosynthesis (Xing et al. 2007). Our results showed that all lasers irradiated at the 1278 J cm^{-2} dose did not change the photo-acclimation capacity of *M. aeruginosa.*

Photosynthetic pigments of *M. aeruginosa* play an important role in photosynthesis. Chl. *a* is the principal pigment that is involved in light absorption and photochemistry (Eullaffroy and Vernet 2003). Carotenoid is considered as an accessory pigment harvesting light and protecting molecules against photo-oxidative damage (Gotz et al. 1999). Our results indicated that laser irradiation could induce a decrease of both Chl. *a* and carotenoid, which may affect the photosynthesis of *M. aeruginosa*. Jiang and Qiu (2005) found that carotenoid/Chl. *a* of *M. aeruginosa* increased with enhanced UV-B irradiation probably as a result of resisting oxidative damage and accelerating the repair of damaged photosynthetic apparatus, while the carotenoid/Chl. *a* ratio decreased with increasing laser irradiation dose, suggesting that the damage caused by laser could not be mediated by regulating photosynthetic pigments, likely due to the high energy density of laser. Besides, a decrease of the ratios indicates that carotenoid is more sensitive than Chl. *a*. In addition, carotenoids are antioxidants for the removal of toxic oxygen species, and a decrease in carotenoids could lead to the impairment of the antioxidant defense system in cells.

PC and APC are antenna pigments assembled in phycobilisomes and are attached to the surface of thylakoids for photosynthesis, which play important roles in funneling light energy to the underlying PS II reaction centers (Glazer 1988). Zhou et al. (2006) reported that degradation of phycobiliproteins is a crucial acclimation response of *M. aeruginosa* under exogenous stress conditions. Our results revealed that both PC and APC contents were reduced, and PC/Chl. *a* and APC/Chl. *a* ratios decreased considerably following laser irradiation. The damage of phycobilisomes caused by laser irradiation might lead to less energy being transferred to the PS II reaction center, resulting in photosynthetic damage and growth inhibition. The changes of PC/Chl. *a* and APC/Chl. *a* ratios also indicated that APC was more sensitive than PC to laser irradiation at 650 nm, which might be because the maximum absorption of APC was close to 650 nm (652 nm), while the maximum absorption of PC was 615 nm (Bennett and Bogorad 1973).

To counteract the toxicity of reactive oxygen species (ROS), cells have developed a set of cellular defense system via the enzymatic and nonenzymatic antioxidants (Hong et al. 2008). SOD and POD are two important enzymatic antioxidants which can scavenge ROS. SOD is considered as a catalyst which converts superoxide anion O_2^- to H_2O_2 and O_2, which is the first line of defense in organisms to remove ROS (Hassan and Scandalios, 1990). H_2O_2 generated during the conversion of O_2^- can be further eliminated by POD and other antioxidants (Apel and Hirt 2004). It has been reported that laser irradiation could enhance enzyme activities of seeds and plants (Chen et al. 2005a; Jamil et al. 2013; Podlesny et al. 2012). Therefore, increasing SOD and POD activities under laser irradiation could be a strategy to protect cells from oxidative damage.

MCs are cyclic heptapeptides produced by cyanobacteria, including *M. aeruginosa*, through nonribosomal peptide synthases. MCs are hepatotoxic and can pose a major threat to drinking water safety. Kaebernick et al. (2000) reported that the damage of the photosynthetic system of *Microcystis* might negatively impact intracellular MC production. Factors including light, simulated microgravity, and organic pollutants can also significantly affect MC production and release (Wang et al. 2007; Wiedner et al. 2003; Xiao et al. 2010). In our study, the detection of extracellular MC in the control group can be attributed to the high culture density (Wiedner et al. 2003). Lower dose irradiation (642 and 1284 J cm^{-2}) did not change intracellular and extracellular MC contents, suggesting that no release of MC from *M. aeruginosa* cells and no degradation of MC occurred. With 1926 J cm^{-2} or higher doses, the intracellular MC content decreased while extracellular MC content increased, and the total MC content in the system showed no significant difference from the control, indicating that MC was released from the cell into the culture medium and no degradation of MC occurred during laser exposure. The release of MC can be attributed to the damage of the *M. aeruginosa* cell wall, as observed in TEM (Fig. 7). Therefore, laser irradiation may have a risk of increasing MC release, but the risk can be minimized by optimizing the exposure time.

The ultrastructural changes of *M. aeruginosa* cells during exposure showed that laser irradiation affect thylakoids first. Chl. *a* and carotenoids are attached to membrane-bound proteins of the thylakoids while phycobilins are attached to the cytosol face of the thylakoids and extend into the cytosol for photosynthesis. Therefore, the function of photosynthetic and respiratory electron transport chain was broken following laser irradiation. Then, other organelles including cyanophycin granules, polyphosphate bodies, and gas vesicles were injured by laser. Thereafter, the cell wall was affected and the permeability of the cell membrane was altered, resulting in the release of MCs. Finally, the nucleoid, cytosol, and inclusions were severely disrupted, and cellular defense and resistance disappeared (Fig. 7d). As a result, the activities of SOD and POD dropped below detection and pigment contents were further deteriorated when receiving 3210 J cm^{-2} irradiation.

The biostimulation effects of laser irradiation on organisms are mainly photochemical, electromagnetic, and thermal effects. Phytoplankton, especially cyanobacteria, is very sensitive to light environment (Chen et al. 2005b). Laser is a specific light of high energy intensity that can be effectively absorbed by phytochromes and cause certain photochemical

reactions (Popve et al. 2007). Phytochrome is a component of photoreceptor system in plant cells, and it is located in the submembrane such as plasmalemma chloroplast membrane (Jamil et al. 2013). After being irradiated by laser, light absorption and photochemical reaction might directly influence the photosynthesis of *M. aeruginosa*. The energy of the excited molecules is transformed into chemical energy for their subsequent physiological activity (Hernández et al. 2015). The activities of related enzymes, which are modulated by phytochrome, could be enhanced. Besides, laser irradiation could induce free radicals like hydroxyl radical, which can react with other molecules (Podlesany et al. 2012). Electromagnetic fields can damage the molecular structure of protein, enzymes, and DNA (Simko 2007). Therefore, the electromagnetic effect of high-intensity laser irradiation could lead to oxidative damage and destroy the molecular structure of cellular pigments, protein, and DNA in the protoplast of the *M. aeruginosa* cell. As a result, the activities of SOD and POD enhanced; the cell wall, cell membrane, thylakoids, and organelles in *M. aeruginosa* cell were destroyed; and the intracellular MC was released after *M. aeruginosa* irradiated by laser.

Additionally, our results showed that temperature of algal suspension irradiated by laser increased significantly, indicating a process of heating the cells. In this way, laser irradiation could cause enhancement of enzyme activities. Some researchers found that laser irradiation could change the thermodynamic parameters of seed and the kinetic equilibrium of seed germination was broken (Hernández et al. 2015; Chen et al. 2005b). Therefore, it is inferred that the effect of laser irradiation on algal suspension may be a combination of photochemical, electromagnetic, and thermal effects.

Conclusions

The present study elucidated the effectiveness and possible mechanisms of laser irradiation on *M. aeruginosa*. Results indicated that laser irradiation can effectively inhibit the growth of *M. aeruginosa*. Assays of photosynthetic activities, pigment contents, enzymatic antioxidant activities, and MC contents combined with TEM imaging demonstrated the progressive damage of physiological functions and destruction of ultrastructure of *M. aeruginosa* cells, which contributed to the inhibition of growth. When exposed to lower-dose laser irradiation, *M. aeruginosa* can take adaptive strategies to cope with the stress while higher doses can destroy the protecting mechanisms and lead to the apoptosis of cells. Effects of laser irradiation on *M. aeruginosa* cells may be due to a combination of photochemical, electromagnetic, and thermal effects. The effectiveness of laser irradiation on *M. aeruginosa* suggested that techniques based on laser may be used for algal bloom control. In the future, further experiments will be performed to study the effectiveness, feasibility, and economical efficiency of using laser-based technology for algal bloom control in eutrophicated waters.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

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