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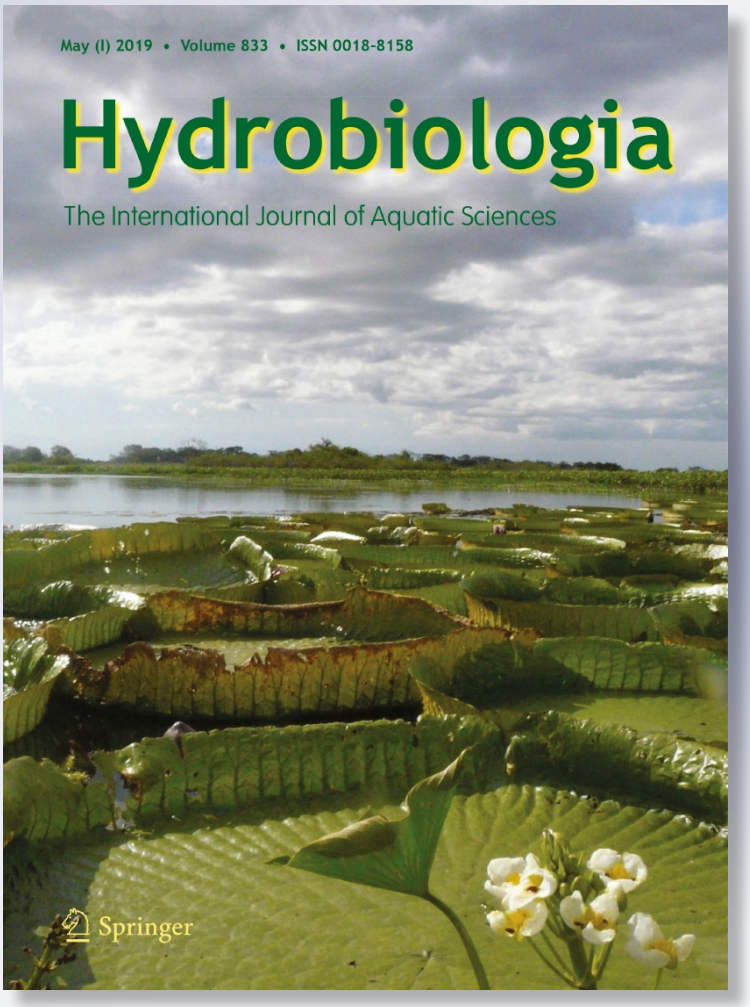

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PRIMARY RESEARCH PAPER

Physiological response of the toxic and non-toxic strains of a bloom-forming cyanobacterium *Microcystis aeruginosa* to changing ultraviolet radiation regimes

Zhiguang Xu · Guang Gao · Bo Tu · Hongjin Qiao · Hongmei Ge · Hongyan Wu

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Abstract *Microcystis aeruginosa*, a common bloom-forming cyanobacterium with both non-toxic and toxic strains, experiences a variable light environment due to buoyancy regulation and the variable mixing of the water columns. However, little is known on how non-toxic and toxic strains respond to changing photosynthetically active radiation (PAR) and ultraviolet radiation (UVR). Here, the non-toxic and toxic strains of *M. aeruginosa* were exposed to simulated solar radiation for 6 h, and their physiological changes were investigated at different irradiance levels of UVR (295–400 nm) in combination with PAR (400–700 nm). Our results showed that UVR at each level resulted in a larger inhibition on the maximum photochemical yield of Photosystem II (PSII) in the toxic strain. The non-toxic strain showed a quicker rise in the non-photochemical quenching when PAR + UVR were below 40.8 + 5.0 W m^{-2} and higher exopolysaccharide content at each radiation level, while the toxic strain exhibited stronger recovery capacity and superoxide dismutase (SOD) activity compared with the non-toxic strain, particularly for cells treated in the presence of UVR. In addition, UVR induced much higher content of microcystin in the toxic strain with the increase of irradiance levels, but decreased it when UVR was higher than 7.3 W m^{-2}. Although UVR led to growth inhibition in both strains, the toxic strain showed much higher specific growth rate under UVR in comparison with the non-toxic strain. Our results indicate that the toxic strain has a competitive advantage relative to the non-toxic strain in a changing light environment with increase of UVR and PAR via stronger antioxidant capacity (higher SOD activity and the synthesis of microcystin) and quicker PSII recovery capacity.

Keywords Cyanobacterium · Exopolysaccharide · Microcystin · Photosystem II · Superoxide dismutase · UVR

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Introduction

Cyanobacteria are the largest and most widely distributed group of photosynthetic prokaryotes on the Earth (Ferreira et al., 2004). Cyanobacterial blooms can occur frequently in both fresh and marine water (Paerl & Otten, 2013; Domingues et al., 2014). These blooms degrade the recreational value of surface water, produce toxins and odorous compounds (van Apeldoorn et al., 2007; Zhang et al., 2010), cause deoxygenation of the water (Dokulil & Teubner, 2000), and lead to fish kills (Bouchard et al., 2006). Bloom-forming cyanobacteria are exposed to UV-B radiation on a mass scale, particularly during the surface bloom and scum formation that can persist for long periods of time (Ding et al., 2013). In recent years, the effects of UV-B radiation on bloom-forming cyanobacteria have received increasing attention (Jiang & Qiu, 2005, 2011; Sommaruga et al., 2009; Hernando et al., 2018; Mloszewska et al., 2018). UV-B radiation affects a number of physiological and biochemical processes in cyanobacteria, such as growth, pigmentation, photosynthesis, and nitrogen fixation (Jiang & Qiu, 2005; Sinha et al., 1995; Yang et al., 2015). Cyanobacteria also have antioxidant systems, including the synthesis of SOD, exopolysaccharide, microcystin, and light recovery capacity to defend themselves from UV-B (Chen et al., 2009; Zeeshan & Prasad, 2009; Jiang & Qiu, 2011; Yang et al., 2015). In addition, there are a few studies showing that the negative effects of UV-B are mitigated when UV-B radiation is combined with PAR (Häder & Sinha, 2005) or UV-A (Häder et al., 2015). For instance, Sicora et al. (2003) demonstrated that the PSII protein repair capacity was enhanced in *Synechocystis* 6803 cells, when UV-B radiation is accompanied by low irradiance visible light. Our recent studies showed that the function of PSII in *Arthrospira platensis* (Nordstedt) Gomont could be maintained under simultaneous exposure to UV-B and low irradiance of visible light (Wu et al., 2011), but the extent of the relative protective effect depended on the irradiance level of PAR and the total radiation that cell received (Liu et al., 2014).

Microcystis aeruginosa Kützing is one of the most notorious bloom-forming cyanobacteria in freshwater (Sabart et al., 2010). Studies have shown that UV-B exposure can damage the water-oxidizing complex, induce the production of reactive oxidants, inhibit the photosynthetic activity and growth, and decrease the microcystin content in *Microcystis* cells (Jiang & Qiu, 2005, 2011; Yang et al., 2014). To date, most studies focused on the impacts of UV-B alone on *Microcystis* (Jiang & Qiu, 2005, 2011; Yang et al., 2014, 2015; Ding et al., 2013; Zhang et al., 2013). In terms of the effect of UV-A on *M. aeruginosa,* Hernando et al. (2018) showed that the photosynthesis inhibition produced by UV-A was 50% higher on average compared to that produced by UV-B corresponding to 66.4–74.4 kJ m^{-2} of UV-B doses when cells were exposed to natural sunlight at Buenos Aires (34°35′S; 58°22′W) during spring and summer in 2014 and 2015. Although UV-B radiation is more effective per energy unit, UV-A is usually responsible for most of the UVR damage as its natural level is much higher (Gao et al., 2009; Hernando et al., 2018).

Like some other bloom-forming cyanobacteria, *Microcystis* has both toxic and non-toxic strains. Toxic *Microcystis* strains possess a suite of microcystin synthesis genes (mcyA–mcyJ), while non-toxic strains do not (Davis et al., 2009). Previous studies have shown that toxic and non-toxic strains of *Microcystis* respond differentially to temperature and nutrients (Davis et al., 2009, 2010), which may lead to dynamic distribution of these two kinds of strains under various environmental conditions. In aquatic environments, *Microcystis* is exposed to a changing light environment due to mixing events as well as vertical migration controlled by its buoyancy regulation (Kromkamp & Walsby, 1990). However, little is known regarding the response of toxic and non-toxic strains of *Microcystis* to changing light conditions, particularly to both PAR and UVR radiation. In addition, the toxic strain shows higher tolerance to UV-B compared to the non-toxic strain but the potential mechanisms remain unclear (Ding et al., 2013; Yang et al., 2015). Based on the previous studies (Chen et al., 2009; Zeeshan & Prasad, 2009; Yang et al., 2015; Gao et al., 2018b), we hypothesize that toxic strain may obtain competitive advantage against non-toxic strain in responding to UVR by its stronger antioxidant system and light recovery capacity. In this study, we exposed *M. aeruginosa* to various UVR intensities in the presence of PAR, measuring superoxide dismutase activity, exopolysaccharide, and microcystin production to test its antioxidant system and monitoring D1 content and PSII activity under

high and low irradiances of PAR or PAR + UVR to test its light recovery capacity.

Materials and methods

Culture conditions and radiation treatments

The unicellular cyanobacteria *M. aeruginosa* FACHB 912 (toxic strain) and 469 (non-toxic strain) were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and grown in BG11 medium (Stanier et al., 1971) at 40 µmol photons m^{-2} s^{-1} PAR (cool white fluorescent light) with a light: dark period of 12:12 h at 25°C. Cells in the exponential growth phase were diluted with fresh medium to achieve an initial concentration of about 5×10^6 - cells ml^{-1}, which was determined using an Automated Cell Counter (Countstar®BioTech, IC1000, China). Three hundred milliliters of culture replicates was dispensed in each UV-transparent quartz tube (inside diameter, 6.4 cm; length, 20 cm) and then maintained in a flow-through water bath for temperature control (25 ± 1°C). The cultures were aerated (0.3 l min^{-1}) and exposed to two radiation treatments: (1) cells receiving PAR + UVR (295–700 nm) in quartz tubes covered with Ultraphan film 295 (UV Opak, Digefra, Munich, Germany); (2) cells receiving only PAR (400-700 nm) in quartz tubes covered with Ultraphan film 395 (UV Opak, Digefra, Munich, Germany). The PAR was set 100, 200, 300, or 400 µmol photons m^{-2} s^{-1} (20.4, 40.8, 61.2, 81.6 W m^{-2}), and the corresponding UVR (295–400 nm) was 2.4, 5.0, 7.3, or 8.2 W m^{-2}, provided by a solar simulator (Sol 1200, Dr. Hönle GmbH, Germany) equipped with a 1000-W xenon arc lamp. The irradiances were measured with a spectroradiometer (PMA 2100, Solar light, USA). Cultures were exposed to these radiation treatments for 6 h, and then cells were collected for physiological and biochemical analysis.

Chlorophyll fluorescence measurement

Chlorophyll fluorescence was determined using a portable pulse amplitude modulated fluorometer (WATER-PAM, Walz, Germany). First, the samples were kept in total darkness for 10 min. Then the minimal fluorescence yield (F_o) was determined by illuminating the cells with a modulated light of low irradiance (< 0.1 µmol photons m^{-2} s^{-1}). Subsequently, a saturating flush (4000 µmol photons m^{-2} s^{-1}) was applied and the maximal fluorescence in the dark-adapted state (F_m) was assessed. After obtaining the steady-state fluorescence (F) by actinic light (50 µmol photons m^{-2} s^{-1}), the minimal fluorescence in the light-adapted state (F_o') was determined by applying a weak far-red light. The maximal fluorescence in the light-adapted state (F_m') was measured using a saturating flush. The maximal efficiency of PSII photochemistry was determined as the ratio of variable to maximal chlorophyll fluorescence (F_v/F_m), where $F_v = (F_m - F_o)$. Non-photochemical quenching (NPQ) was calculated as $NPQ = (F_m - F_m')/F_m'$.

UVR-induced inhibition rates on F_v/F_m were calculated as follows: inhibition rate = $(P_P - P_U)/P_P \times 100\%$, where P_P and P_U are the F_v/F_m of *M. aeruginosa* under PAR alone and PAR plus UVR, respectively.

Immunoblotting for D1 quantification

Cells were harvested on glass fiber filters (0.4 µm effective pore size, 25 mm diameter, binder free glass fiber, Whatman), which were immediately flash-frozen in liquid nitrogen and stored at − 80°C for later protein analyses by immunoblotting. Total proteins were extracted by two thawing/sonicating rounds in denaturing extraction buffer (Brown et al., 2008). The total protein concentration was determined using a Lowry protein assay kit (Biorad-DC Assay). Western blots were performed for D1, a core reaction center protein in Photosystem II. One µg of total protein was loaded on 6% to 12% acrylamide gels. Electrophoresis was run for 60 min at 200 V and the proteins were transferred to a PVDF membrane. After membrane blocking, primary antibody against the C-terminal part of D1 (Agrisera, 1: 50,000) was applied (Wu et al., 2011, 2012a; Yuan et al., 2018), followed by an anti-rabbit secondary antibody coupled with horseradish peroxidase. The blots were scanned and analyzed with Tanno Gis Analyzer software (Tanno 5200, China).

Assay of superoxide dismutase (SOD) activity

SOD activities were tested using SOD Assay Kits (Nanjing Jiancheng Biological Engineering Company,

China). One unit of SOD activity was defined as the amount of enzyme which resulted in a 50% inhibition of the rate of nitro-blue tetrazolium reduction at 560 nm. The absorbance at 560 nm was measured using a spectrophotometer (UV-2700, Shimadzu). The activity was expressed on a basis of protein content. Total protein content of the supernatant was determined using an enhanced BCA Protein Assay Kit (Beyotime Institute of Biotechnology, China).

Exopolysaccharide (EPS) contents

Samples were harvested and centrifuged at $5000\times g$ for 15 min at 4°C. The supernatants were filtered through 0.45-μm filters (47 mm, Whatman). The filtrated solutions were dialyzed with deionized water at 4°C overnight (Vincenzini et al., 1990). The EPS content in each supernatant was quantified spectrophotometrically (UV-2700, Shimadzu) by the phenol–sulfuric acid method using glucose as a standard (Dubois et al., 1956). Exopolysaccharide concentrations were measured in four replicates and normalized to cell number.

Microcystin (MC) analysis

Cells were harvested and centrifuged at $10{,}000\times g$ for 10 min at 4°C. The pellets were suspended in PBS solution, then frozen in liquid nitrogen, and thawed at 4°C to rupture the cells. To obtain the solution containing intracellular MC, the ruptured cells were centrifuged at $10{,}000\times g$ for 10 min at 4°C. The supernatants were diluted with Milli-Q water and detected with an enzyme-linked immunosorbent assay (ELISA) method. ELISA was performed using the QuantiPlate™ Kit for Microcystin (EnviroLogix, USA) according to the manufacturer's instructions. The absorbance was measured at 450 nm using a multimode reader (BioTek Instruments, Inc. USA). We also analyzed the non-toxic strain with the same ELISA method and the result was negative. The validation of ELISA has been proved (Sheng et al., 2006), which shows a high relativity of more than 99% with high-performance liquid chromatography. The quantitative detection range of ELISA was 0.01–3 μg l^{-1} (Sheng et al., 2006) and our results (0.09–0.3 μg l^{-1}) fall into this range.

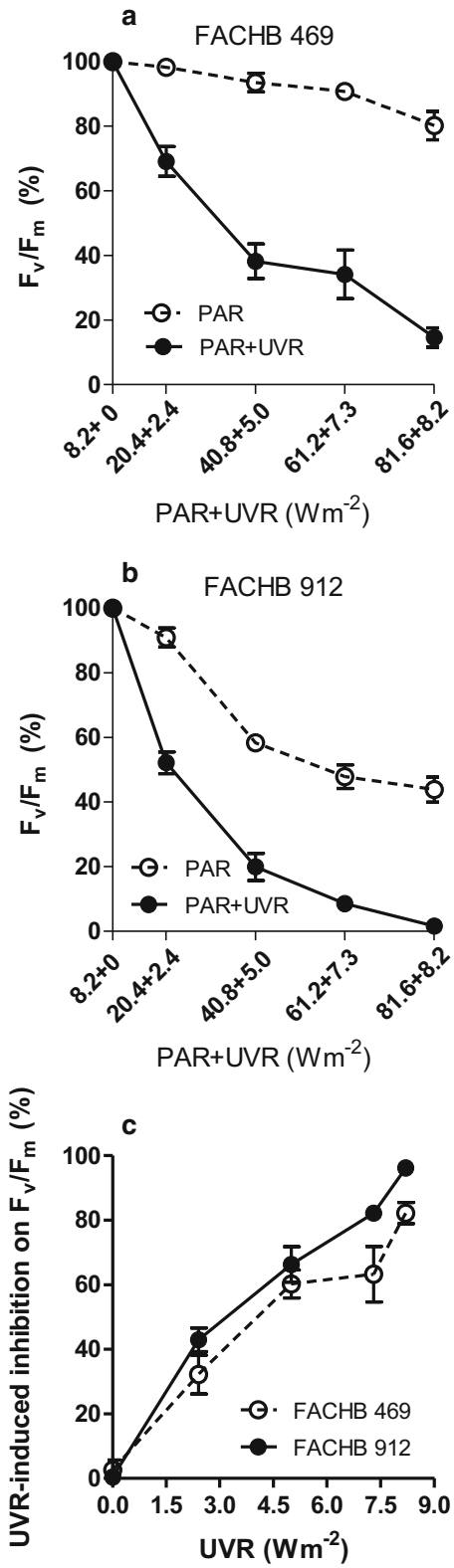
UVR-induced production in microcystin was calculated as follows: microcystin (%) = $(M_U - M_P)/M_P \times 100\%$, where M_U and M_P are the microcystin content under PAR plus UVR and PAR alone, respectively.

Low light recovery

To determine the recovery capability of *M. aeruginosa*, an upward light shift and recovery experiment was performed. We shifted the cells grown at 40 μmol photons m^{-2} s^{-1} of PAR to higher level of PAR (200 μmol photons m^{-2} s^{-1}, i.e., 40.8 W m^{-2}) or PAR + UVR (40.8 + 5 W m^{-2}) radiation treatment for 6 h, and then returned them to their initial growth light of 40 μmol photons m^{-2} s^{-1} of PAR for 1-h recovery. Based on the previous experiments, Fv/Fm for the toxic strain decreased to about 20% of the initial value measured at growth light of 8.2 W m^{-2} PAR when cells were exposed to 40.8 + 5 W m^{-2}, and thus it is a good irradiance point to be used for upward light shift. The same cell concentration, volume, and quartz tubes as the experiments above were used. Samples were taken prior to the onset of high light exposure (plotted as time 0), after 6-h high light exposure, and after 0.5-h and 1-h recovery periods for chlorophyll fluorescence measurement. All experiments were carried out in 4 replicates.

Specific growth rate

To assess the adaptation of *M. aeruginosa* cells to UV radiation, long-term experiment was performed. Three hundred milliliters of culture replicates with initial cell density of about 6.0×10^6 cells ml^{-1} was incubated in UV-transparent quartz tubes (inside diameter, 6.4 cm; length, 20 cm) under a 12:12 L:D cycle with an irradiance of 40 μmol photons m^{-2} s^{-1} at 25°C for at least 7 days. During this incubation period, they were exposed to PAR (200 μmol photons m^{-2} s^{-1}, i.e., 40.8 W m^{-2}) or PAR + UVR (40.8 + 5 W m^{-2}) radiation treatments for 2 h per day from 13:00 to 15:00. For the consistence of this study, we still used 40.8 + 5 W m^{-2} (PAR and UVR) to assess the adaptation of *M. aeruginosa* to UVR. In our pre-experiments, the non-toxic strain of *M. aeruginosa* only survived for several days when they were exposed to UVR for longer time (4–6 h) every day, and we thus chose 2-h exposure to UVR per day. Similar treatments could be found in other research on *M. aeruginosa* (Jiang & Qiu, 2005). The cell number



◀**Fig. 1** Responses of PSII maximum photochemical yield (F_v/F_m) versus irradiance level to PAR and PAR + UVR treatments in the non-toxic FACHB 469 (**a**) and toxic strain FACHB 912 (**b**) of *M. aeruginosa*, and UVR-induced inhibition on F_v/F_m in both strains (**c**). The data are shown after normalized to the F_v/F_m value at growth light of 8.2 W m^{-2} PAR. Data are mean ± standard error ($n = 4$); some error bars are within symbols

was determined every day using an Automated Cell Counter (Countstar®BioTech, IC1000, China) which can count cells in colonial form (Yu et al., 2019). The final culture volume for each tube was 265 ml. The specific growth rate (μ) was calculated from the slopes of linear regressions of ln (cell concentration) versus day for individual cultures over the range of linearity.

Statistical analyses

Data were analyzed using the software SPSS v.21. The data from each treatment conformed to a normal distribution (Shapiro–Wilk, $P > 0.05$) and the variances could be considered equal (Levene's test, $P > 0.05$). Three-way ANOVA was conducted to analyze the effects of radiation treatment, irradiance level, and strain on F_v/F_m, NPQ, relative D1 protein amount, SOD activity, and EPS content. Two-way ANOVA was conducted to assess the effects of radiation levels and strain on UVR-induced inhibition of F_v/F_m and specific growth rate, as wells as the effects of radiation treatment and irradiance levels on microcystin content. Tukey's Honestly Significant Difference (Tukey HSD) was conducted for three-way and two-way ANOVA's post hoc comparison. Repeated measures Analysis of Variance (RM-ANOVA) was conducted to analyze the effect of exposure time on F_v/F_m and Bonferroni was conducted for post hoc investigation. The threshold value for determining statistical significance was $P < 0.05$.

Results

Photoinhibition of the photochemical yield of PSII

The maximum photochemical yield of PSII, represented by F_v/F_m, was progressively inhibited with the increase of irradiance levels under PAR and PAR + UVR treatments after 6-h exposure in both *M.*

aeruginosa strains (Fig. 1, $F_{(4,60)} = 1026.081$, $P < 0.001$). In the non-toxic strain FACHB 469, the highest irradiance level of 81.6 W m^{-2} PAR led to the decrease of F_v/F_m to 80% of the initial value measured at growth light of 8.2 W m^{-2} PAR (Fig. 1a, Tukey HSD, $P < 0.05$). In contrast, F_v/F_m in the toxic strain FACHB 912 further decreased to 44% of the initial value (Fig. 1b, Tukey HSD, $P < 0.05$). The presence of UVR (8.2 W m^{-2}) accelerated the decrease of F_v/F_m to 15% of the initial in the non-toxic strain FACHB 469 and almost caused a complete inhibition in the toxic strain FACHB 912 ($F_{(4,60)} = 43.552$, $P < 0.001$). UVR-induced inhibition continuously increased from the low to high irradiance level (Fig. 1a, $F_{(1,30)} = 422.280$, $P < 0.001$), particularly in the toxic strain FACHB 912 ($F_{(1,30)} = 40.597$, $P < 0.001$).

Non-photochemical quenching (NPQ) was induced for both strains (Fig. 2, $F_{(4,60)} = 525.830$, $P < 0.001$), but the patterns of NPQ versus radiation were different between two strains ($F_{(1,60)} = 1026.081$, $P < 0.001$). Post hoc Tukey HSD comparison ($P < 0.05$) showed that NPQ in the non-toxic strain FACHB 469 had a quicker rise with radiation (8.2–40.8 W m^{-2} for PAR) and reached a plateau at the radiation level of 40.8 W m^{-2} (PAR). By contrast, NPQ in the toxic strain FACHB 912 continuously increased with radiation until the highest radiation level. UVR treatment induced much higher NPQ than PAR in both strains ($F_{(1,60)} = 754.203$, $P < 0.001$).

Changes of the D1 protein contents

The amount of D1 protein was progressively decreased when cells were exposed to PAR and PAR + UVR treatments from low to high irradiance levels (Fig. 3, $F_{(4,60)} = 147.219$, $P < 0.001$). The presence of UVR resulted in a further degradation of D1 protein ($F_{(1,60)} = 12.323$, $P = 0.001$). Post hoc Tukey HSD comparison ($P = 0.05$) showed that in the non-toxic strain, PAR and PAR + UVR treatments at the highest irradiance level led to a decrease in D1 protein to 61.0% and 38.4% of the initial value measured at growth light, respectively. In contrast, the D1 protein in the toxic strain decreased to great extent after 6-h exposure to the highest irradiance level, reaching to 27.9% and 15.4% of the initial value under PAR and PAR + UVR treatments, respectively.

Changes of the SOD activity

The activity of SOD was stimulated by PAR and PAR + UVR treatments as the irradiance increased from the growth light to 81.6 (PAR) and 8.2 (UVR) W m^{-2} (Fig. 4, $F_{(4,60)} = 72.893$, $P < 0.001$). The presence of UVR induced higher SOD activity in both strains ($F_{(1,60)} = 14.514$, $P < 0.001$). In addition, radiation treatment and irradiance level had an interactive effect on SOD activity ($F_{(4,60)} = 3.122$, $P = 0.021$); SOD activity under PAR + UVR had a quicker increase when PAR increased from 8.2 to 40.8 W m^{-2}, while the increase was slower when

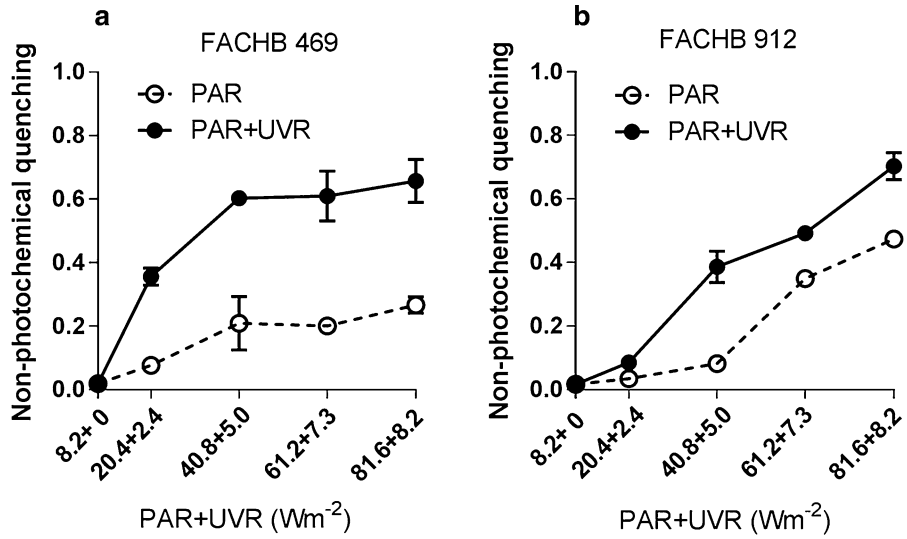


Fig. 2 Responses of non-photochemical quenching (NPQ) versus irradiance level to PAR and PAR + UVR treatments in the non-toxic FACHB 469 (**a**) and toxic strain FACHB 912 (**b**) of *Microcystis aeruginosa*. Data are mean ± standard error ($n = 4$); some error bars are within symbols

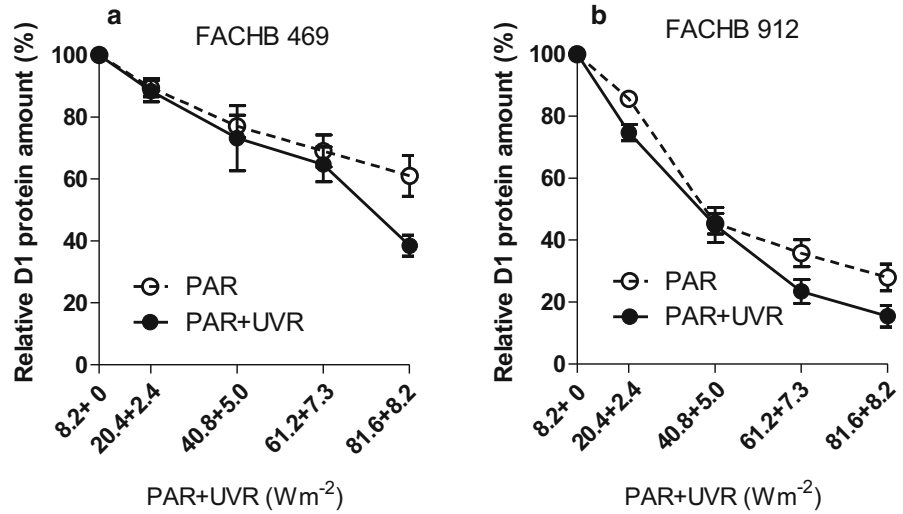


Fig. 3 Changes of the D1 protein at different irradiance levels under PAR and PAR + UVR treatments in the non-toxic FACHB 469 (**a**) and toxic strain FACHB 912 (**b**) of *Microcystis aeruginosa*. The relative data, which were determined by absolute data divided by the value measured in the cells cultured at growth light of 8.2 W m^{-2} PAR, are shown in this figure. Data are mean ± standard error (n = 4); some error bars are within symbols

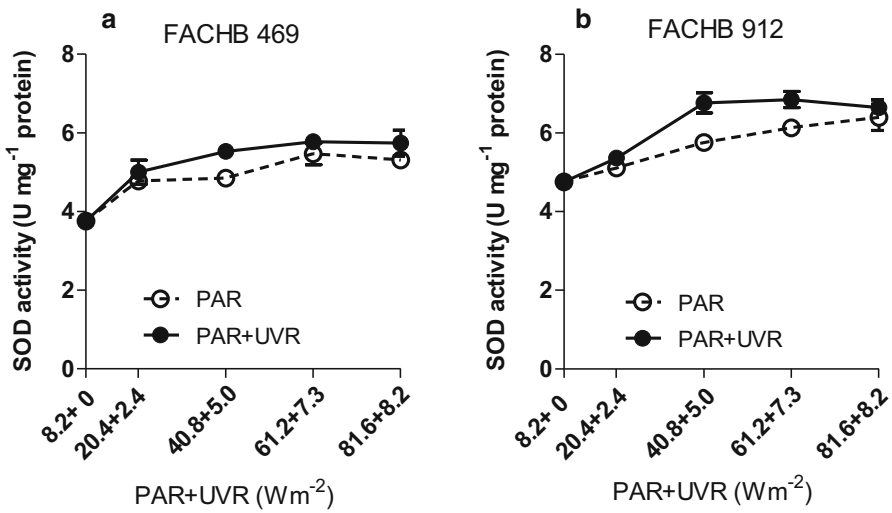


Fig. 4 Changes of superoxide dismutase (SOD) activity at different irradiance levels under PAR and PAR + UVR treatments in the non-toxic FACHB 469 (**a**) and toxic strain FACHB 912 (**b**) of *Microcystis aeruginosa*. Data are mean ± standard error (n = 4); some error bars are within symbols

PAR exceeded 40.8 W m^{-2} compared to that under PAR. The toxic strain FACHB 912 showed the maximum induction of SOD activity of 6.6 U mg^{-1} protein under the two radiation treatments, which is about 1.2 times larger than that in the non-toxic strain FACHB 469 (Tukey HSD, $P < 0.05$).

Changes of EPS contents

EPS was induced for both strains as the irradiance increased from the growth light to 81.6 (PAR) and 8.2 (UVR) W m^{-2} (Fig. 5, $F_{(4,60)} = 839.818$, $P < 0.001$), and the toxic strain FACHB 912 showed a relatively lower induction of EPS compared to the non-toxic strain FACHB 469 ($F_{(1,60)} = 239.651$, $P < 0.001$). At the highest irradiance level, the contents of EPS under PAR + UVR for the toxic strain and non-toxic strain were 1.13 ± 0.05 and 1.40 ± 0.10 pg $cell^{-1}$, respectively. PAR + UVR induced more EPS compared to PAR only for both strains ($F_{(1,60)} = 219.601$, $P < 0.001$).

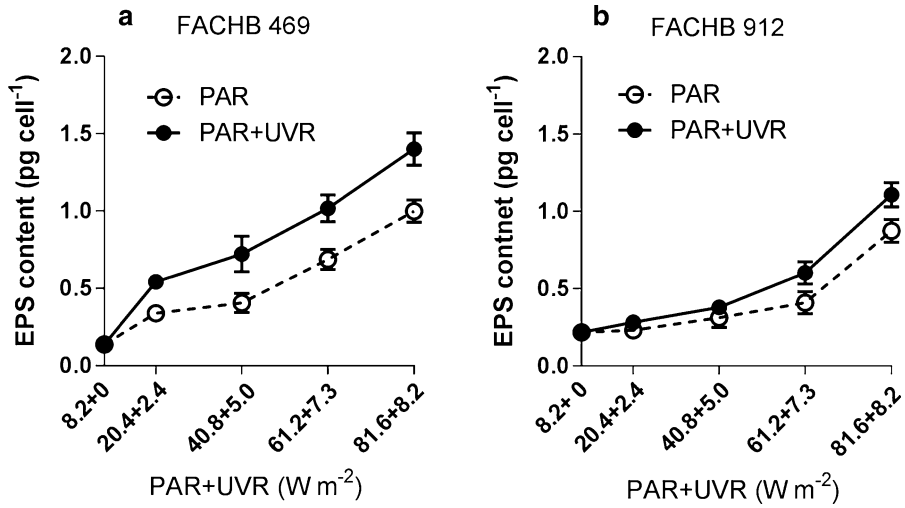


Fig. 5 Changes of the exopolysaccharide (EPS) content at different irradiance levels under PAR and PAR + UVR treatments in the non-toxic FACHB 469 (**a**) and toxic strain FACHB 912 (**b**) of *Microcystis aeruginosa*. Data are mean ± standard error (n = 4); some error bars are within symbols

Changes of MC production

Increasing PAR stimulated the production of MC in the toxic strain FACHB 912 (Fig. 6a, $F_{(4,20)}$ = 55.042, $P < 0.001$), and the presence of UVR accelerated the production of MC ($F_{(1,20)}$ = 23.127, $P < 0.001$). UVR-induced production of MC increased to 50% of the content in cells exposed to the corresponding PAR treatment when UVR reached 7.3 W m^{-2}, but then decreased with the further increase of UVR irradiance to 8.6 W m^{-2}, being 44% of the PAR-induced MC induction (Fig. 6b).

Upward light shift and recovery experiment

M. aeruginosa cells grown at 40 µmol photons m^{-2} s^{-1} of PAR were exposed to 200 µmol photons m^{-2} s^{-1} (i.e., 40.8 W m^{-2}) of PAR or PAR + UVR (40.8 + 5 W m^{-2}) for 6 h and then returned to the initial growth condition for a 1-h recovery period (Fig. 7). In the non-toxic strain FACHB 469 (Fig. 7a), F_v/F_m changed with incubation time ($F_{(3,18)}$ = 246.934, $P < 0.001$) and radiation treatment had an interactive effect with incubation time ($F_{(3,18)}$ = 166.670, $P < 0.001$). Specifically, post hoc Bonferroni comparison (P = 0.05) showed that F_v/F_m slightly decreased under PAR treatment, showing

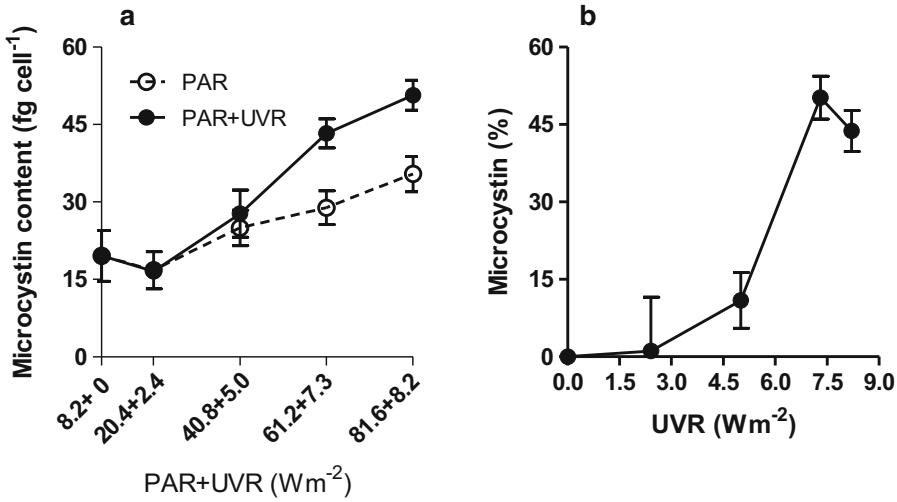


Fig. 6 The content of microcystin at different irradiance levels under PAR and PAR + UVR treatments in the toxic strain FACHB 912 of *Microcystis aeruginosa* (**a**), and UVR-induced production in microcystin compared to PAR alone (**b**). Data are mean ± standard error (n = 4); some error bars are within symbols

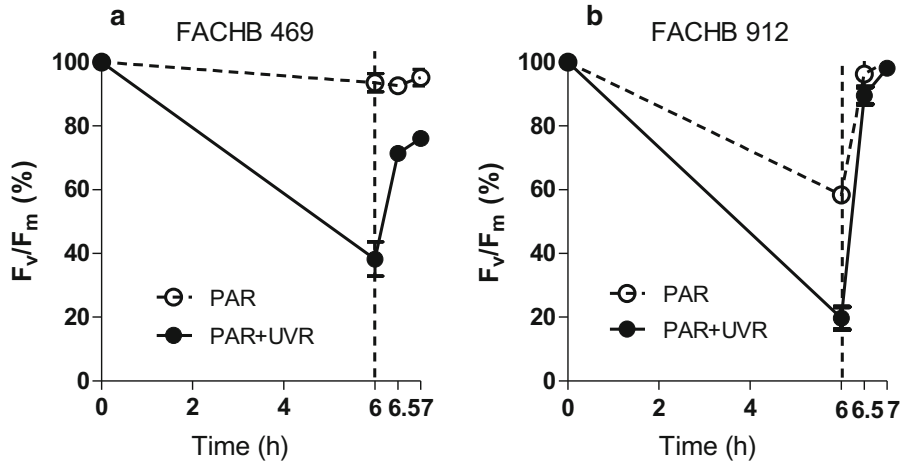


Fig. 7 Responses of PSII maximum photochemical yield (F_v/F_m) versus time to PAR and PAR + UVR treatments in the non-toxic strain FACHB 469 (**a**) and toxic strain FACHB 912 (**b**) of *Microcystis aeruginosa*. Cells grown at PAR irradiance of 40 µmol photons m^{-2} s^{-1} were exposed to PAR and PAR + UVR treatments with irradiance level of PAR 200 µmol photons m^{-2} s^{-1} (40.8 W m^{-2}) and UVR 5.0 W m^{-2} for 6 h, and then allowed to recover at growth light of 40 µmol photons m^{-2} s^{-1} for 1 h. The relative data, which were determined by absolute data divided by the values measured in the cells cultured at growth light of 8.2 W m^{-2} PAR, are shown in this figure. Data are mean ± standard error (n = 4); some error bars are within symbols

only 6% lower than the initial at time 0, and remained constant even when shifted to growth light of 40 µmol photons m^{-2} s^{-1} PAR (Fig. 7). In contrast, PAR + UVR treatment resulted in a significant decrease of F_v/F_m to 38% of the initial, and F_v/F_m increased to 76% of the initial after shifted to growth light for 1 h (Bonferroni, $P < 0.05$). In the toxic strain FACHB 912 (Fig. 7b), incubation time and radiation treatment also interacted on F_v/F_m ($F_{(3,18)} = 81.868$, $P < 0.001$). PAR and PAR + UVR treatments led to a marked reduction of F_v/F_m to 58% and 20% of the initial, respectively (Bonferroni, $P < 0.05$). On the other hand, cells recovered quickly during the shift to growth light for half an hour, particularly in cells treated by PAR + UVR. F_v/F_m increased to 98% of the initial after the cells were returned to growth light for 1 h for both PAR and PAR + UVR treatments.

Changes of the growth rate

The specific growth rate (µ) was inhibited significantly by UVR in both strains ($F_{(1,12)} = 155.541$, $P < 0.001$, Fig. 8), particularly for the non-toxic strain ($F_{(1,12)} = 155.541$, $P < 0.001$); UVR reduced the specific growth rate of the toxic strain FACHB 912 by 11%, while it was a decrease of 35% for the non-toxic strain FACHB 469. This result indicates that the toxic strain is more tolerant to UVR.

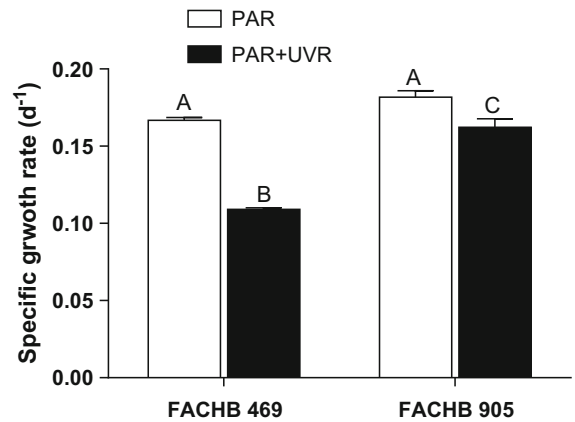


Fig. 8 Specific growth rate of the non-toxic strain FACHB 469 and toxic strain FACHB 912 under PAR and PAR + UVR treatments. Cells were cultured under a 12:12 L:D cycle with an irradiance of 40 µmol photons m^{-2} s^{-1} at 25°C for at least 7 days. During this culture period, they were exposed to PAR (200 µmol photons m^{-2} s^{-1}, i.e., 40.8 W m^{-2}) or PAR + UVR (40.8 + 5 W m^{-2}) radiation treatments for 2 h per day. Data are mean ± standard error (n = 4)

Discussion

In this study, the toxic and non-toxic *M. aeruginosa* strains were affected to different extents by the PAR and PAR + UVR treatments at various irradiance levels. Photosynthetic activity in both strains decreased with increasing PAR as shown by the decline of the PSII quantum yield (F_v/F_m), and the presence of UVR led to a further decrease of F_v/F_m, causing larger photoinactivation of PSII. The similar pattern was also found in diatoms (Gao et al., 2009; Wu et al., 2012b; Gao et al., 2018b). It has been shown that visible and UVR photons inactivate PSII via non-interacting mechanisms. UVR primarily damages the Mn cluster of water oxidation in the PSII complex, which can be followed by additional damage induced by visible light in the PSII structure (Hakala et al., 2005; Ohnishi et al., 2005; Gao et al., 2018b). However, the two spectral ranges interacted in the process of PSII repair; when UVR was accompanied with low irradiance visible light, the PSII protein repair capacity was enhanced (Sicora et al., 2003; Wu et al., 2011). In the present study, the content of D1 protein was decreased when cells were transferred from lower to higher irradiance levels of PAR and the addition of UVR led to a further decrease. Our previous study on *Arthrospira platensis* showed that supplementation with PAR (< 2.6 W m^{-2}) enhanced the D1 protein content in cells exposed to 7.9 W m^{-2} UVR (Liu et al., 2014). The relative higher irradiance level of PAR (> 20.4 W m^{-2}) used here might lead to the insignificantly ameliorating effect on UVR-induced damage since *M. aeruginosa* is a typically low light-adapted organism (Kardinaal et al., 2007; Deblois & Juneau, 2012), and a similar result was also found in the study on *Synechocystis* 6803 (Sicora et al., 2003). Upon a sudden increase in irradiance for both PAR and PAR + UVR, high non-photochemical quenching (NPQ) was induced in both strains, indicating that *M. aeruginosa* cells can dissipate excess light energy through NPQ to reduce overexcitation of their photosystems. It was assumed that cyanobacteria do not possess the energy quenching mechanism based on the xanthophyll cycle (Campbell et al., 1998), but recently, Wang et al. (2011) have demonstrated that a fast quenching component of NPQ, independent from state transition or photoinhibition and induced by the conformational changes in photosynthetic pigment proteins on thylakoid membranes (PPPTM), is an important responding mechanism for *Microcystis* to high-irradiance light (Wang et al., 2012). The presence of UVR might act upon the conformation of PPPTM, leading to high NPQ induction.

Overexcitation of PSII can lead to the production of reactive oxygen species (Müller et al., 2001; Rastogi et al., 2010; Weisz et al., 2017), causing damage to the photosynthetic apparatus (Nishiyama et al., 2006; Gao et al., 2018a). The superoxide dismutase (SOD) is an important antioxidant in nearly all living cells, which is responsible for converting superoxide radicals into either ordinary molecular oxygen or hydrogen peroxide. In this study, the activity of SOD was greatly stimulated in both *M. aeruginosa* strains exposed to PAR + UVR, particularly for the toxic strain, indicating the high efficiency of cells in defending against a sudden increased irradiance. The stimulating effect of UVR on SOD activity was also reported by Hernando et al. (2018). However, the antioxidant response of *M. aeruginosa* seemed not to be enough to remove the reactive species since photoinactivation still occurred in both strains in this study.

Cyanobacterial extracellular polysaccharides (EPS) are considered to facilitate the aggregation of cyanobacteria (Reynolds, 2007; Gan et al., 2012). It has been showed that colonial *M. aeruginosa* has more total carbohydrate in cells and excretes more soluble carbohydrate into the environment compared to disaggregated *M. aeruginosa* (Zhang et al., 2007). The aggregated morphology is helpful to reduce UVR damage by shading. In addition, EPS can prevent DNA strand breaks and lipid peroxidation by effectively eliminating ROS induced by UV-B radiation in *M. vaginatus* Gomont ex Gomont, exhibiting a direct protective function against UV-B (Chen et al., 2009). In this study, the extracellular polysaccharide (EPS) production was stimulated by UV radiation particularly for non-toxic strain. This should be a defending response of *M. aeruginosa* to UVR.

For the toxic strain, the presence of UVR induced higher MC content with the increase of irradiance levels as compared with PAR alone. Our results disagreed with that observed by Yang et al. (2015), who found that UV-B radiation decreased the MC content. The discrepancy appears to be related to the way of UV radiation treatments. In their study, the combination of UV-B (the maximum emission at 312 nm) and PAR was exploited, while in our study, a full spectrum of radiation (UV-B + UV-A + PAR,

295–700 nm) was used to mimic the natural solar radiation. The presence of UV-A might stimulate the photo-reactivation (Häder & Sinha, 2005), reducing and repairing the damages produced by UV-B in the process of MC synthesis.

The previous studies have demonstrated that MC synthesis may contribute to the higher fitness of the toxin-producing strain under UV radiation due to its functions in protein-modulating metabolites and dealing with oxidative stress (Phelan & Downing, 2011; Zilliges et al., 2011; Meissner et al., 2015). It was also reported that MC might serve as an allelochemical for toxin-producing *M. aeruginosa* to increase its competitive advantage against other algae (Schatz et al., 2005; Yang et al., 2014). Increased MC content might be very important for the toxic strain to be advantageous in unfavorable conditions (Gan et al., 2012).

In addition to high-irradiance exposure, recovery capacity of *M. aeruginosa* under low irradiance level after high irradiance exposure was also investigated in this study. The toxic strain had much lower F_v/F_m during the 6-h exposure to increased PAR and UVR but showed faster recovery from the photoinhibition stress compared to non-toxic strain. It was found that under high visible light condition toxic *M. aeruginosa* cells could accumulate more energy despite PSII photoinhibition (Deblois & Juneau, 2012). The decline of PSII activity under UVR might be a kind of strategy for the toxic strain to avoid the damage of excessive energy brought by UVR. In addition, it might indicate that the toxic *M. aeruginosa* cells possess very efficient mechanisms to recover from light stress similar to what they can experience in natural environment, particularly when increased UVR is present.

The previous studies have documented that the toxic strain of *M. aeruginosa* is more tolerate to UV-B, with a lower inhibition of UV-B on growth of the toxic *M. aeruginosa* compared to the non-toxic strain (Ding et al., 2013; Yang et al., 2015). However, the potential mechanisms remain unknown. Our study also showed that the toxic strain had a higher growth rate during the exposure to UVR. We deem that stronger antioxidant capacity (including higher SOD activity and the synthesis of MC) and quicker PSII recovery capacity endow the toxic strain with a higher tolerance to UVR. Although the resources investment in MC production could lead to fewer resources available for other cellular functions, such as the synthesis of EPS and D1 protein, it seems that the benefit from MC outweighs the drawbacks it brings about. Furthermore, the toxic strain may produce more D1 protein when cells were transferred to low PAR irradiance from UVR because MC production was reduced and more resources were available for D1 protein synthesis under that condition. That might explain the higher recovery capacity of toxic strain after being transferred to low PAR irradiance. The strong antioxidant system under UVR exposure and quick recovery capacity under low PAR are extremely important for *M. aeruginosa* to acclimate to the changing environment as it experiences such light changes in the field due to mixing events and vertical migration itself (Kromkamp & Walsby, 1990; Jiang & Qiu, 2011). It is worth noting that the quicker growth rate for toxic strain resulted in bigger self-shading effect, which also contributed to the higher tolerance to UVR (Garcia-Pichel, 1994).

Conclusion

Our study investigated the physiological response of non-toxic and toxic strain of *M. aeruginosa* to UVR in the presence of PAR. The non-toxic strain showed quicker thermal dissipating responses and higher EPS production after 6 h of PAR + UVR exposure. In contrast, the toxic strain had more efficient antioxidant systems and stronger recovery capacity and showed higher specific growth rate under PAR + UVR. In addition, the toxic strain could improve its competitive capacity by producing more toxic compounds under stressful environments. These findings provide helpful information in understanding different responses of non-toxic and toxic strains of *M. aeruginosa* to a fluctuating light environment. Thus, the mechanisms described in this work demonstrate that the toxic strain has a higher tolerance to UVR compared to the non-toxic strain. More in situ studies should be performed to confirm the conclusions made here.

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