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Influence of ofloxacin on photosystems I and II activities of *Microcystis aeruginosa* and the potential role of cyclic electron flow

Chunnuan Deng,[1,2] Xiangliang Pan,[2,*] and Daoyong Zhang[2,3]

Key Lab of Plateau Lake Ecology & Global Change, College of Tourism and Geographic Science, Yunnan Normal University, 768 Juxian Street, Kunming, China,[1] Laboratory of Environmental Pollution and Bioremediation, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, 818 Beijingnan Road, Urumqi 830011, China,[2] and State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, 46 Guanshui Road, Chinese Academy of Sciences, Guiyang, China[3]

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Pollution with antibiotics poses a great risk to aquatic ecosystems. Although some toxic effects of antibiotics on photosystem II (PSII) have been documented, their toxicity to photosystem I (PSI) is still unclear. In this study, effects of ofloxacin on activities of both PSI and PSII of *Microcystis aeruginosa* (Kützing) Kützing were investigated. Exposure to 0.1 mg L^{-1} ofloxacin led to increases in contents of chlorophyll *a* and carotenoids and photosynthetic activity of *M. aeruginosa*. PSI activity and its electron transport were not affected by 0.1 mg L^{-1} ofloxacin. When *M. aeruginosa* was exposed to ≥10 mg L^{-1} ofloxacin, the electron transport rates of PSI and PSII, the yield of cyclic electron flow (CEF) and the contribution of linear electron flow (LEF) to PSI decreased whereas Y(NA) (limitation of donor side of PSI) and Y(NO) (the quantum yield of non-regulated energy dissipation in PSII) significantly increased. CEF had a significant contribution to alleviating the inhibitory effect of ofloxacin on PSI of *M. aeruginosa* treated with low concentrations of ofloxacin. The protective role CEF for tolerance of PSI to the toxicity of ofloxacin decreased with increasing ofloxacin concentration.

[**Key words:** Chlorophyll fluorescence; Cyanobacteria; Photosystem I; Photosynthesis; Toxicity]

Widespread use and improper disposal of antibiotics may pose hazardous risks to ecosystems (1–3). Annual global consumption of antibiotics was estimated to be 100,000–200,000 tons (4). In HK/Pearl River Delta (PRD) region in China, around 15,770 tons of antibiotics were used for human in 2004 (5). Most antibiotics are poorly adsorbed by organisms and excreted unchanged into environments (6,7). Furthermore, most antibiotics tested are not easily biodegradable under aerobic conditions (6,7) and they are continuously introduced into the environment. High concentrations of various antibiotics have been frequently detected in hospital and residential effluents.

Recently, toxic effects of antibiotics on microalgae have been reported (8–10). The toxicity of antibiotics to microalgae includes their acute toxicity (1,11), damage to antioxidant system (12), inhibition of cell growth (13) and photosynthesis (14–16). Isidori et al. reported that algae were sensitive to six antibiotics with EC_{50} values ranging from 0.002 to 1.44 mg L^{-1} (17). Antibiotics could significantly inhibit the physiological processes including primary photochemistry and antioxidant system of algae (18). They can reduce cell growth, chlorophyll content and photosynthetic rate of cyanobacteria (13,19). One recent study shows that fluoroquinolone inhibits PSII activity and enzymatic activity related to photosynthetic electron transport in PSII (15). Some studies showed that photosystem I (PSI) was also a damage target to various environmental stressors (20,21), being usually less affected than PSII (21). Although some effects of antibiotics on PSII have been documented (22), effects of antibiotics on PSI and the regulation mechanism between PSII and PSI of cyanobacteria are still unclear.

The quinolones are one of the most important groups of synthetic antibiotics. Quinolones have been detected at μg L^{-1} in hospital effluents (6). The relatively high concentration (35.5 μg L^{-1}) of ofloxacin was found in hospital and residential effluents (23). It was reported that ciprofloxacin at higher concentrations than 1.0 mg L^{-1} significantly inhibited electron transport in PSII of *Selenastrum capricornutum* (19). Ofloxacin, one of most widely used quinolones, has genotoxicity and low biodegradability (24). Therefore, its pollution of aquatic environments may have adverse effects on aquatic organisms.

Microcystis aeruginosa (Kützing) Kützing, one of the most common cyanobacteria, has been found to be more sensitive to most antibiotics than green algae (1) and is often used as a model microorganism for testing toxicity of chemicals (18,25). Chlorophyll fluorescence technique has been proved to be useful to study effects of environmental stresses on photosynthesis (26,27). In the present study, the powerful Dual-PAM-100 chlorophyll fluorometer (Walz, Germany) was used for examining the effects of ofloxacin on PSII and PSI activities and regulation mechanism between PSII and PSI in *M. aeruginosa*.

* Corresponding author. Tel./fax: +86 991 7823156.
E-mail address: panxl@ms.xjb.ac.cn (X. Pan).

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MATERIALS AND METHODS

Culture of *M. aeruginosa* *M. aeruginosa* (FACHB-905), purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, was incubated in BG-11 medium (28) at 25°C under 30 μmol photons m^{-2} s^{-1} illumination with a 12/12 h light/dark period. Cells at exponential phase at a density of 9.6×10^7 cells mL^{-1} were transferred into 10 × 10 mm plastic cuvettes for analysis of pigment content and PSII and PSI activities.

Ofloxacin treatment Ofloxacin ($C_{18}H_{20}FN_3O_4$, purity >98%) was purchased from Hefei BoMei Biotechnology Co. Ltd., China. Ofloxacin was dissolved in distilled water and stored at 4°C prior to use. All experiments were conducted in a sterilized cabinet. 0.1 mL of distilled water or various concentrations of ofloxacin solution was added into cell suspension to obtain a series of final nominal ofloxacin concentrations of 0, 0.1, 1, 10, 50 and 100 mg L^{-1}. Because antibiotics were detected at mg L^{-1} levels in some lake water and fish hatcheries (29,30), effects of ofloxacin at concentrations from 100 μg L^{-1} to 100 mg L^{-1} were examined. The treatment without addition of ofloxacin was used as the control. The cell suspensions were cultured at 25°C under 30 μmol photons m^{-2} s^{-1} illumination. After 24 h of incubation, the cell samples were used to measure contents of pigments and activities of both PSII and PSI.

Measurement of contents of pigments After 24 h of exposure to ofloxacin, cyanobacterial cells were harvested by centrifugation at 8000 rpm for 5 min at 4°C. The pigments were extracted with 80% acetone for 24 h at 4°C in the dark. Then the extracts were centrifuged at 8000 rpm at 4°C for 5 min. Absorption of the supernatant was measured with a spectrophotometer (UV2800, Unico, Shanghai, China). Contents of chlorophyll (Chl) *a* and carotenoids were calculated (31).

Measurement of polyphasic fast fluorescence induction The polyphasic fast fluorescence induction was performed using a double-modulation fluorometer (FL3500, PSI, Brno, Czech). The cells used for Chl *a* fluorescence measurements were dark-adapted for 15 min before each test. The polyphasic fluorescence transient was measured with a 1 s multiple turnover flash and recorded every 10 μs for the first 2 ms and every 1 ms up to 1 s. The values of F_V/F_M, the maximal PSII photochemical efficiency were calculated according the fast fluorescence induction curves.

Measurement of quantum yield of PSI and PSII PSI and PSII activities of *M. aeruginosa* were measured simultaneously with a Dual-PAM-100 system (Heinz Walz GmbH, Effeltrich, Germany) (32). All samples were dark-adapted for 15 min prior to measurements. F_0, the minimum fluorescence, was detected by a measuring light at low intensity. A 300 ms saturating pulse of 10,000 μmol photons m^{-2} s^{-1} was applied to determine the maximum fluorescence (F_m). The maximal change in P700 signal (P_m) was measured using a saturation pulse after far-red light (33).

After determination of F_0, F_m and P_m, the slow induction curves were recorded with the routine program of the Dual-PAM-100 software (34). Actinic light at 30 μmol m^{-2} s^{-1} was applied. A saturating pulse with duration of 300 ms was applied every 20 s after the onset of the actinic light to determine the maximum fluorescence signal (F_m') and maximum $P700^+$ signal (P_m') under the actinic light. The slow induction curve was recorded for 120 s to achieve the steady state of the photosynthetic apparatus, and then the actinic light was turned off. The P700 signal (P) was recorded just before a saturation pulse then briefly after onset of a saturation pulse (P_m'), when the maximum P700 oxidation was observed. Finally, P_0 was determined at the end of the 1 s dark interval following each saturation pulse. The signals P and P_m' were detected referencing to P_0.

The quantum yields of PSI and PSII were measured by saturating pulses during the process of slow induction curve (33,35): $Y(II) = (F_m'-F)/F_m'$, $Y(NPQ) = F/F_m'-F/F_m$, $Y(NO) = F/F_m$, where F is the steady state fluorescence, Y(II) is the effective photochemical quantum yield of PSII, Y(NPQ) is the regulated energy dissipation, and Y(NO) is the non-regulated energy dissipation; $Y(I) = (P_m'-P)/P_m$, $Y(ND) = (P-P_0)/P_m$, $Y(NA) = (P_m-P_m')/P_m$, where Y(I) is effective photochemical quantum yield of PSI, Y(ND) is the quantum yield of non-photochemical energy dissipation in reaction centers due to PSI donor side limitation, and Y(NA) is the quantum yield of non-photochemical energy dissipation of reaction centers due to PSI acceptor side limitation.

Calculation of cyclic electron flow and linear electron flow The relation between cyclic electron flow (CEF) and linear electron flow (LEF) was assessed by the ratios of Y(CEF)/Y(I), Y(CEF)/Y(II) and Y(II)/Y(I). The quantum yield of CEF was the difference between Y(I) and Y(II): Y(CEF) = Y(I)–Y(II) (36). Y(CEF)/Y(I), Y(II)/Y(I) and Y(CEF)/Y(II) indicate the contribution of CEF to Y(I), the contribution of LEF to Y(I), and the ratio of the quantum yield of CEF to LEF, respectively. The ratio of Y(II)/Y(I) also presented the distribution of quantum yield between two photosystems.

Calculation of electron transport rates in PSI and PSII Electron transport rates (ETRs) in PSI and PSII, i.e., ETR(I) and ETR(II), were recorded during the measurement of the slow induction curve and defined and calculated using the Dual-PAM software. The responses of electron transport in PSI and PSII to increasing irradiation were measured by the recording the Rapid Light Curves (RLCs). The RLC consists of the electron transport responses to eleven increasing irradiances (PAR 0, 14, 30, 61, 103, 224, 347, 539, 833, 1295 and 1960 μmol photons m^{-2} s^{-1}) for 30 s. The following RLC parameters were derived using the exponential function (37). α, the initial slope of RLC of ETR(I) or ETR(II), reflected the quantum yield (38) of PSI or PSII; ETR_{max}, the maximal electron transport rates in PSI or PSII; I_k, the index of light adaptation of PSI or PSII (i.e., determined from the interception point of the alpha value with the maximum photosynthetic rate), was calculated as ETR_{max}/α (37).

Statistics analyses Each treatment was replicated three times. Means and standard deviation (S.D.) were calculated. The statistical significance between treatments and control were performed by one-way ANOVA (SPSS V16.0) through least significant difference (LSD) test.

RESULTS

Effects of ofloxacin on pigments content Treatment with 0.1 mg L^{-1} ofloxacin increased Chl *a* and carotenoids contents of *M. aeruginosa* by 23% and 21%, respectively. However, Chl *a* and carotenoids contents decreased by 42% and 35% at 10 mg L^{-1} ofloxacin ($p < 0.05$) (Fig. 1). After exposure to high concentrations of ofloxacin, Chl *a* and carotenoids contents decreased with the increase of ofloxacin concentration.

Effects of ofloxacin on the maximal PSII photochemical efficiency F_V/F_M values, which may be the most sensitive parameter in chlorophyll fluorescence, were calculated according to the fast fluorescence induction curves (Fig. 2). Exposure to 50–100 mg L^{-1} ofloxacin induced significant inhibition of the maximal PSII photochemical efficiency. Treatment with 50 and 100 mg L^{-1} ofloxacin decreased F_V/F_M by 75% and 97% compared to control, respectively.

Effects on quantum yield of PSI and PSII Activities of both PSI (Fig. 3A) and PSII (Fig. 3B) were affected after 24 h of exposure to ofloxacin. Y(I) increased slightly at ≤10 mg L^{-1} ofloxacin but decreased with increasing ofloxacin concentration from 50 to 100 mg L^{-1}. Y(ND) decreased slightly at ≤10 mg L^{-1} ofloxacin but increased by 230% at ≥50 mg L^{-1} ofloxacin. The value of PSI acceptor side limitation [Y(NA)] decreased from 0.097 for control to 0.015 ($p < 0.01$) and 0.040 ($p < 0.05$) at 50 and 100 mg L^{-1} ofloxacin, respectively. Y(II) decreased as ofloxacin concentration increased, accompanied with an increase of Y(NO) ($p < 0.001$ at ≥50 mg L^{-1} ofloxacin). When ofloxacin concentration increased to 50 and 100 mg L^{-1}, Y(II) dropped from 0.19 for control to 0.04 and zero, respectively ($p < 0.001$). Y(NO) increased by 18% at 50 mg L^{-1} ofloxacin with respect to the control.

Effects on CEF and LEF Y(CEF) of *M. aeruginosa* did not change significantly when cells were treated with ≤10 mg L^{-1} ofloxacin but markedly decreased as ofloxacin concentration

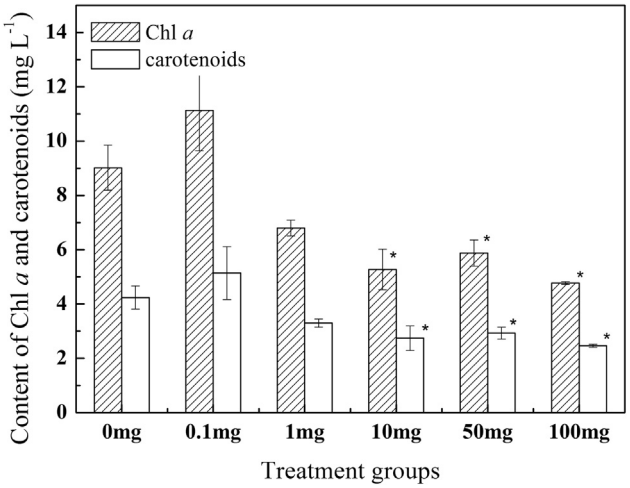

FIG. 1. Contents of Chl *a* and carotenoids of *M. aeruginosa* untreated and treated with various concentrations of ofloxacin for 24 h ($n = 3$) (* $p < 0.05$).

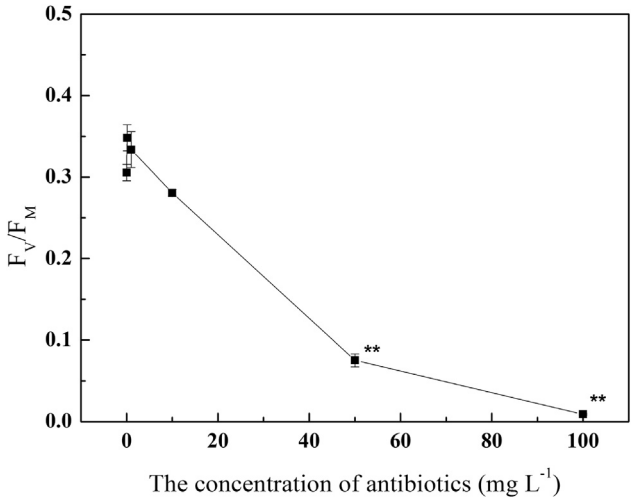

FIG. 2. Values of maximum efficiency of PSII photochemistry (F_V/F_M) of *M. aeruginosa* untreated and treated with various concentrations of ofloxacin for 24 h ($n = 3$) (** $p < 0.01$).

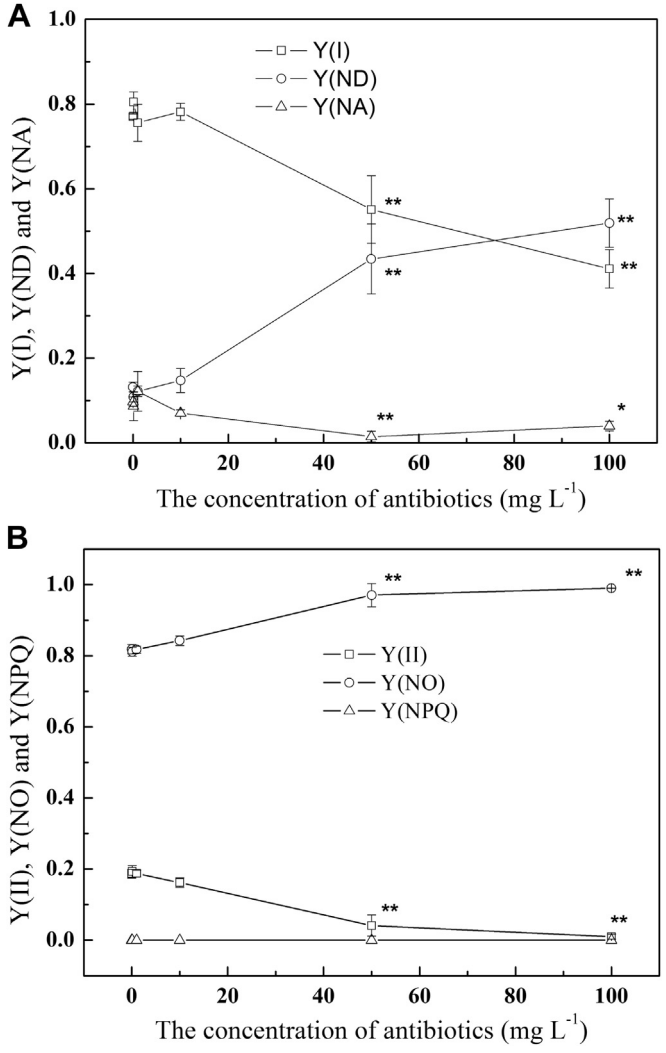

FIG. 3. (A) Y(I) (quantum yield of PSI), Y(ND) (non-photochemical energy dissipation due to donor-side limitation), and Y(NA) (non-photochemical energy dissipation due to acceptor side limitation of PSI) of *M. aeruginosa* exposed to various concentrations of ofloxacin for 24 h; (B) Y(II) (quantum yield of PSII), Y(NO) (non-regulated energy dissipation) and Y(NPQ) (regulated energy dissipation of PSII) of *M. aeruginosa* exposed to various concentrations of ofloxacin for 24 h ($n = 3$) (** $p < 0.01$).

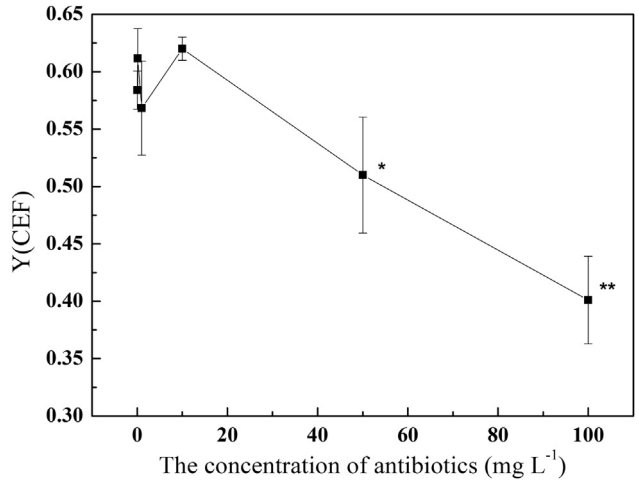

FIG. 4. Y(CEF) (the quantum yield of cyclic electron flow) of *M. aeruginosa* exposed to various concentrations of ofloxacin for 24 h (** $p < 0.01$).

increased from 50 to 100 mg L^{-1} ($p < 0.05$ at 50 mg L^{-1}, $p < 0.001$ at 100 mg L^{-1}) (Fig. 4). Y(CEF) decreased by 12% and 31% at 50 and 100 mg L^{-1} ofloxacin, respectively. Y(CEF)/Y(II) ($p < 0.05$) increased greatly at 50 and 100 mg L^{-1} ofloxacin while the ratio of Y(II)/Y(I) significantly decreased by 71% and 92% at 50 and 100 mg L^{-1} ofloxacin, respectively (Fig. 5).

Effects on electron transport of PSI and PSII $ETR_{max}(II)$ increased a little when the cells were exposed to ≤1.0 mg L^{-1} ofloxacin and then significantly dropped with increasing ofloxacin concentration (Table 1). $ETR_{max}(II)$ were almost fully inhibited at 50 mg L^{-1} ofloxacin. $ETR_{max}(I)$ decreased as ofloxacin concentration increased from 10 to 100 mg L^{-1}. It decreased to 35% of the control at 50 mg L^{-1} ofloxacin. Compared with the control, $I_k(II)$ markedly decreased by 63% at 50 mg L^{-1} ofloxacin. $I_k(I)$ changed slightly in the presence of ≤1 mg L^{-1} ofloxacin and markedly decreased with increasing ofloxacin concentration from 10 to 100 mg L^{-1}. $a(II)$ and $a(I)$ significantly decreased when the cells were exposed to 50 mg L^{-1} or higher concentrations of ofloxacin (Table 1).

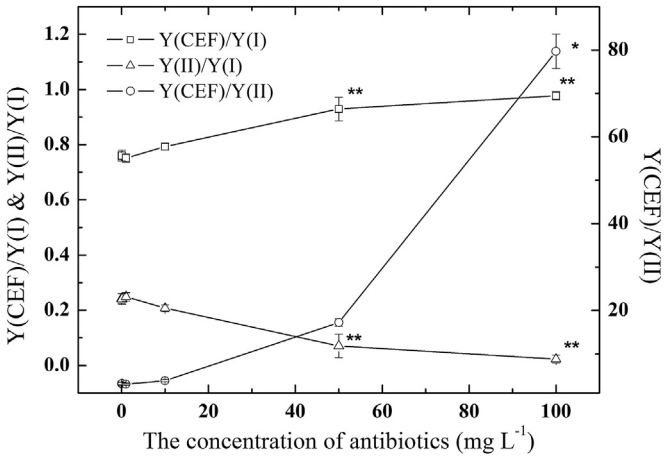

FIG. 5. Y(CEF)/Y(I) [ratios of the contribution of cyclic electron flow (CEF) to Y(I)], Y(CEF)/Y(II) (the ratio of the quantum yield of cyclic electron flow to linear electron flow) and Y(II)/Y(I) (the distribution of quantum yield between two photosystems) of *M. aeruginosa* exposed to various concentrations of ofloxacin for 24 h (** $p < 0.01$).

TABLE 1. Parameters of electron transport in PSII and PSI, which were derived from the rapid light curves of ETR_{II} and ETR_{I} ($n = 3$).

Ofloxacin (mg L^{-1})	RLC parameters of PSII			RLC parameters of PSI		
	α(II) (e^- photon^{-1})	ETR_{max}(II) (μmol e^- m^{-2} s^{-1})	I_k(II) (μmol photon m^{-2} s^{-1})	α(I) (e^- photon^{-1})	ETR_{max}(I) (μmol e^- m^{-2} s^{-1})	I_k(I) (μmol photon m^{-2} s^{-1})
0	0.08 ± 0.01	58.70 ± 8.45	696.80 ± 85.69	0.33 ± 0.01	178.13 ± 16.82	532.70 ± 59.88
0.1	0.09 ± 0.01	65.10 ± 13.67	743.70 ± 89.00	0.33 ± 0.02	177.70 ± 14.70	536.43 ± 71.59
1	0.08 ± 0.01	70.45 ± 3.32	849.40 ± 86.59	0.33 ± 0.01	178.77 ± 5.02	538.75 ± 4.73
10	0.08 ± 0.01	53.90 ± 10.30	696.63 ± 72.95	0.34 ± 0.01	152.03 ± 6.79*	451.13 ± 11.06
50	0.03 ± 0.00***	6.3 ± 3.25***	254.85 ± 55.3**	0.23 ± 0.05*	62.25 ± 7.84***	273.90 ± 18.38**
100	–	–	–	0.20 ± 0.05**	35.25 ± 4.45***	201.75 ± 74.39***

α is the initial slope of rapid light curves (RLCs) of ETR; ETR_{max} is the maximum electron transport rates; I_k indicates the light saturation. Significant levels between the control and treatments with various concentrations of ofloxacin are indicated by asterisks ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Data for PSII of cells treated with 100 mg L^{-1} ofloxacin were too low to be calculated.

DISCUSSION

Treatment with 0.1 mg L^{-1} ofloxacin increased Chl *a* and carotenoids contents (Fig. 1), suggesting that low concentration of ofloxacin may promote the synthesis of Chl *a* and carotenoids and be beneficial for photosynthesis of *M. aeruginosa*. Some previous studies also reported that algal growth was stimulated in the presence of a low concentration of some antibiotics (39,40). Suzuki et al. (39) found that 0.1 mg L^{-1} procaine could stimulate cell division. Ten mg L^{-1} or higher concentrations of ofloxacin reduced Chl *a* and carotenoids contents. Degradation of algal pigments after exposure to high level of antibiotics was frequently reported (41). Liu et al. reported that contents of Chl *a* and carotenoids of freshwater algae *S. capricornutum* decreased when it was exposed to 2 mg L^{-1} and higher concentrations of ciprofloxacin (19). Li et al. reported that 1.5 mg L^{-1} ofloxacin was toxic to algae (42). In the present study, reduction of Chl *a* and carotenoids contents was slightly affected by cell number, since the cell number slightly decreased after exposure to >10 mg L^{-1} ofloxacin (data were not shown). The decrease of Chl *a* content implies a decrease in the antenna size of photosynthetic reaction center complexes (43). Carotenoids pigment is important for non-photochemical quenching (NPQ) and dissipating excess energy under stress to prevent damage to PSII (44). The increase of carotenoids content at 0.1 mg L^{-1} ofloxacin suggests dissipation of excess energy to prevent damage and the decrease of carotenoids content at more than 10 mg L^{-1} ofloxacin indicates ofloxacin at these concentrations induces damage to PSII.

Some recent studies show that photosynthesis is perturbed by fluoroquinolone antibiotics (45). Aristilde et al. (15) found that the quinolone ring and secondary amino group typically present in fluoroquinolone antibiotics may mediate their actions as quinone site inhibitors in PSII, a key enzyme in photosynthetic electron transport. Ciprofloxacin interferes with energy transfer from excited antenna chlorophyll molecules to PSII reaction centers and delays the kinetics of photoreduction of the primary quinone acceptor (15). The F_V/F_M for cyanobacteria is usually underestimated by the influence of cellular phycobiliprotein level and state transitions, but F_V/F_M still reflects the relative potential quantum efficiency of PSII of cyanobacteria. In the present study, F_V/F_M showed a significant decrease after exposed to high levels of antibiotics (Fig. 2), which was in agreement with some early studies (18). The decrease of F_V/F_M may be the result of the increased proportion of Q_B-non-reducing PSII reaction centers (46,47), and higher levels of antibiotics may damage PSII reaction centers. Quantum yield of PSII [Y(II)] was significantly inhibited by 50 mg L^{-1} and higher concentrations of ofloxacin. Electron transport in PSII [ETR_{max}(II)] was almost fully inhibited at 50 mg L^{-1} ofloxacin. The inhibition of PSII activity of cyanobacteria by antibiotics has been reported previously (18,48). In the present study, inhibition of PSII was accompanied by an increase of Y(NO) (Fig. 3). The increase of Y(NO) also reflects the increase of fraction of closed PSII centers and the inability of PSII to protect itself against damage (32). It suggests the regulation mechanism of non-photochemical dissipation of energy did not work well and the excessive excitation energy could not be efficiently dissipated into harmless heat at high concentrations of ofloxacin (49). The decreased I_k(II) for ofloxacin treated *M. aeruginoga* implies that high concentrations of ofloxacin triggered the photoinhibition of PSII at light intensities lower than untreated condition (50). Decrease of I_k(II) was also observed for *M. aeruginoga* exposed to heavy metals (24).

Low concentration of ofloxacin (0.1 mg L^{-1}) had slight positive effect on PSI activity of *M. aeruginosa* (Fig. 3), while high concentrations (≥50 mg L^{-1}) of ofloxacin significantly inhibited PSI activity. Furthermore, the result that Y(I) was less inhibited than Y(II) indicates that PSI is a little more resistant to ofloxacin than PSII. Huang et al. (51) reported the stability of PSI of higher plant under chilling stress. Inhibition of PSI of *M. aeruginosa* by 50 mg L^{-1} and higher concentrations of ofloxacin was associated with the considerable increase of Y(ND) and decrease of Y(NA) (Fig. 3), implying that the decrease of quantum yield of PSI was mainly due to the limitation of donor side of PSI. Increase of Y(ND) represents increased fraction of oxidized P700 lacking donors from PSII (51) and inefficiency of PSII, reflected by the lowered ETR_{max}(II) and I_k(II). The inefficient light absorption in PSII causes low rate of PSII charge separation, which does not match the capacity of PSI and results in the elevated Y(ND) (32,52). The present study shows a significant increase of Y(ND) when the cells were treated with ≥50 mg L^{-1} ofloxacin, indicating that PSI in *M. aeruginosa* was still physiologically healthy and well-regulated (53) and the restricted photochemistry of PSI was owing to inefficient light absorption in PSII. The decreased I_k(I) for samples treated with high concentrations of ofloxacin suggests that that ofloxacin triggered the photoinhibition for PSI and the adaptability of PSI to light decreased (50,54).

When ofloxacin concentration increased from 50 to 100 mg L^{-1}, quantum yield of cyclic electron flow [Y(CEF)] and Y(II)/Y(I) decreased (Fig. 4) whereas Y(CEF)/Y(I) and Y(CEF)/Y(II) increased (Fig. 5). CEF plays an important role in protecting PSII and PSI against environmental stress. A few studies showed that stimulation of CEF was essential for the protection of PSI against stress of Cr(VI) and other stresses (34,51,55). Y(CEF) increased at low concentrations of ofloxacin but decreased at high concentrations of ofloxacin. In the present study, at low concentration of ofloxacin, the PSI functions healthily and electrons can be smoothly transferred from PSI to PQ to prevent the damage to PSI. At high concentrations of ofloxacin, this kind of protection mechanism around PSI was broken (56). The decease of Y(II)/Y(I) with increasing ofloxacin concentration indicates the decrease of the contribution of linear electron flow to PSI. Reduction of LEF from PSII to PSI could help to reduce the damage to PSI (57).

The present study had quantified the effects of ofloxacin on cyanobacteria. Significant inhibitory effects on photosynthetic activities of both PSII and PSI in *M. aeruginosa* were observed at higher

concentrations of ofloxacin (>10 mg L^{-1}). PSII of *M. aeruginosa* was more sensitive to ofloxacin than PSI. The protection mechanism around PSI (CEF) was stimulated in the presence of low concentrations of ofloxacin but weakened at higher concentrations of ofloxacin. These results show that ofloxacin at mg L^{-1} level has acute toxicity to the cyanobacteria. However, the chronic toxicity of antibiotics at μg L^{-1} level to algae needs further study.

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