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Toxic effects of erythromycin on photosystem I and II in *Microcystis aeruginosa*

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Abstract

Environmental pollution by antibiotics poses a potential ecological risk to aquatic photosynthetic organisms. In the present study, toxic effects of erythromycin on PSI and PSII were investigated in cyanobacteria culture medium of *Microcystis aeruginosa*. The activity and electron transport of both photosystems were affected by erythromycin in a concentration-dependent manner. The quantum yield of PSII (Y_{II}) was reduced at 0.1 mg L^{-1} of erythromycin, while the quantum yield of PSI (Y_I) significantly decreased at concentration of 5–25 mg L^{-1}. The decline of Y_{II} was accompanied by an increase of nonregulated energy dissipation (Y_{NO}). At 10 mg L^{-1} of erythromycin, Y_{II} decreased by 55%, while Y_{NO} increased by 18%. The decrease of Y_I induced by erythromycin was caused by donor-side limitation of PSI (Y_{ND}). Y_{ND} was markedly enhanced with elevated erythromycin concentration. At 10 mg L^{-1} of erythromycin, Y_I and Y_{NA} (PSI acceptor-side limitation) decreased by 8 and 82%, respectively, while Y_{ND} rose by 314%. The quantum yield of cyclic electron flow increased significantly at 0.1–1 mg L^{-1} of erythromycin; it decreased but remained higher than that of the control at 5–25 mg L^{-1} of erythromycin. The contribution of cyclic electron flow to Y_I, and to linear electron flow rose significantly with the increasing erythromycin concentration. The maximum values of electron transport rates in PSII and PSI decreased by 71 and 24.3%, respectively, at 25 mg L^{-1} of erythromycin. Compared with the untreated control, the light saturation of PSII and PSI decreased significantly with increasing erythromycin concentration. We showed that concentrations of erythromycin $\geq$ 5 mg L^{-1} could exert acute toxicity to cyanobacteria, whereas the chronic toxicity caused by concentrations of ng or μg L^{-1} needs further research.

Additional key words: chlorophyll fluorescence; nonphotochemical quenching; photoinhibition.

Introduction

Antibiotics are widely used in human medicine and animal agriculture. Annual global consumption of antibiotics was estimated to be 100,000–200,000 tons (Wise 2002). In Hong Kong/Pearl River Delta region of China, around 15,770 tons of antibiotics were used for human purposes in 2004 (Richardson *et al.* 2005). Most antibiotics are excreted into aquatic environments without degradation and they finally reach surface waters (Kümmerer 2009a,b).

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Abbreviations: CEF – cyclic electron flow; ETR – electron transport rate; ETR_I – electron transport rate in PSI; ETR_{II} – electron transport rate in PSII; $ETR_{max(I)}$ – the maximum electron transport rate in PSI; $ETR_{max(II)}$ – the maximum electron transport rate in PSII; $I_{k(I)}$ – the light saturation of PSI; $I_{k(II)}$ – the light saturation of PSII; LEF – linear electron flow; NPQ – nonphotochemical quenching; RLC – rapid light curves; Y_{CEF} – the quantum yield of cyclic electron flow; Y_{CEF}/Y_I – the contribution of cyclic electron flow to Y_I; Y_{CEF}/Y_{II} – the ratio of the quantum yield of CEF to LEF; Y_I – effective photochemical quantum yield of PSI; Y_{II} – the effective photochemical quantum yield of PSII; Y_{II}/Y_I – the distribution of quantum yield between two photosystems; Y_{NA} – nonphotochemical energy dissipation due to acceptor-side limitation; Y_{ND} – nonphotochemical energy dissipation due to donor-side limitation; Y_{NO} – nonregulated energy dissipation; Y_{NPQ} – regulated energy dissipation; α_I – the initial slope of RLC of ETR_I; α_{II} – the initial slope of RLC of ETR_{II}.
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Although environmental concentrations of antibiotics range from ng L^{-1} to μg L^{-1} in most waters, their impact on aquatic organisms cannot be ignored (Stoichev *et al*. 2011, González-Pleiter *et al*. 2013, Pinckney *et al.* 2013). Concentrations of antibiotics can reach mg L^{-1} in water of some lakes, in water of fish hatcheries, and in effluents from pharmaceutical factories (Scribner *et al.* 2004, Fick *et al*. 2009). Since around 30–90% of excreted antibiotics are active (Sarmah *et al*. 2006) and most antibiotics are nonbiodegradable and they are continually released into the water environment (Kümmerer 2009a,b), persistent pollution by antibiotics in lakes and rivers has posed a potential ecological risk to aquatic photosynthetic organisms (Halling-Sørensen 2000, Isidori *et al.* 2005, Park and Choi 2008, Santos *et al*. 2010).

Algae and cyanobacteria are primary producers that occupy the lower levels in a food chain; any change in their population can profoundly affect the aquatic ecosystem. They respond quickly to environmental changes and are usually used for aquatic toxicological assessment of pollutants. Recently, toxic effects of antibiotics on microalgae have been reported (Stoichev *et al*. 2011, González-Pleiter *et al*. 2013, Pinckney *et al.* 2013), including acute toxicity (Holten-Lützhøft *et al*. 1999, Halling-Sørensen 2000), influence on antioxidant systems (Nie *et al*. 2013), and inhibition of photosynthesis (Pan *et al.* 2008, 2009; Aristilde *et al*. 2010). Isidori *et al*. (2005) reported that algae were sensitive to six antibiotics with EC_{50} (antibiotics concentration causing 50% population growth inhibition) values ranging from 0.002 to 1.44 mg L^{-1}. Antibiotics can reduce growth rate, chlorophyll content, and photosynthetic rate of cyanobacteria (Liu *et al.* 2011). Antibiotics could significantly inhibit physiological processes including primary photochemistry and antioxidant system of algae (Yang *et al.* 2013). Exposure to antibiotics can slow down electron transport on both donor and acceptor side of PSII, block the electron transfer from Q_A to Q_B in PSII of *Synechocystis* sp. (Pan *et al.* 2008). Aristilde *et al.* (2010) reported that fluoroquinolone antibiotics inhibit enzyme in photosynthetic electron transport and the activity of reaction center in PSII. Toxic effects of antibiotics on PSII have been well documented in cyanobacteria or algae (Pan *et al*. 2008, Aristilde *et al*. 2010, Liu *et al*. 2011). PSI was less sensitive than PSII to various environmental stressors (Singh *et al.* 2012), but it was also a target site (Singh *et al.* 2012, Belatik *et al.* 2013). Effects of antibiotics on PSI and inter-relationship between PSII and PSI remain unclear and need further research.

The macrolides are broad-spectrum antibiotics against many gram-positive bacteria. Macrolides were shown to be most harmful to the algae (Isidori *et al.* 2005). Macrolide antibiotics have been detected at concentrations of ng L^{-1} to μg L^{-1} in surface water (Alder *et al.* 2004, Lin and Tsai 2009) and at 75 μg L^{-1} in groundwater in Taiwan (Lin and Tsai 2009). Discharge of pharmaceutical waste water without a proper treatment may lead to their accumulation in some sites (Isidori *et al.* 2005). Erythromycin is one of the most commonly used macrolide antibiotics in both human medicine and veterinary practice. Erythromycin is the most frequently detected antibiotics in 139 US stream sites (Kolpin *et al*. 2002), and in some European surface waters and sediments (Zuccato *et al.* 2001). IC_{50} (50% inhibition concentration) of erythromycin was 0.2 mg L^{-1} for alga, *Pseudokirchneriella subcapitata* (Nie *et al.* 2013) and it significantly affected the photosynthesis of this species (Liu *et al.* 2011). Erythromycin is very toxic to algae, such as *Anabaena* CPB4337 and *P. subcapitata*, although the sensitivity of algae to erythromycin may vary with species (Isidori *et al.* 2005, Liu *et al.* 2011, González-Pleiter *et al*. 2013). Therefore, evaluation of the potential ecological impact of erythromycin on algae or cyanobacteria is important for understanding the aquatic ecological risk of antibiotics pollution.

Microcystis aeruginosa, one of the most common cyanobacteria, has been frequently used as a model microorganism for testing toxicity of pollutants (Wang *et al.* 2012), because it has been found to be more sensitive to many antibiotics than other algae (Halling-Sørensen 2000, González-Pleiter *et al*. 2013). In the present study, the *Dual-PAM-100* chlorophyll fluorometer was used to probe the acute toxic effects of erythromycin on PSII and PSI activities and regulation mechanism between PSII and PSI in *M. aeruginosa*.

Materials and methods

Culture: *M. aeruginosa* (FACHB-905) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. The cyanobacteria cells were cultivated in BG-11 medium (Stanier *et al.* 1971) at 25°C and illumination of 30 μmol(photon) m^{-2} s^{-1} with a 12h/12h light/dark photoperiod. Cells in the exponential growth phase were transferred into 10 × 10 mm plastic cuvettes for treatment with antibiotics.

Erythromycin treatment: Erythromycin ($C_{37}H_{67}NO_{13}$, purity > 99%) was purchased from *BioDee BioTech Corporation Ltd*. (Beijing, China). Erythromycin was dissolved in deionized water and diluted to the desired concentrations. Deionized water of 0.1 mL or various concentrations of erythromycin were added into the cell suspension samples to obtain the final erythromycin concentrations of 0, 0.1, 1, 5, 10, and 25 mg L^{-1}. Because antibiotics were detected at mg L^{-1} in environment (Scribner *et al*. 2004, Fick *et al.* 2009), the effect of erythromycin was examined at concentrations from 100 μg L^{-1} to 25 mg L^{-1}. The samples containing no erythromycin were used as a control. After 12 h of exposure to erythromycin, the algae samples were used to measure photosystem activities.

Measurement of quantum yield of PSI and PSII: Quantum yield of PSI and PSII of *M. aeruginosa* were measured simultaneously with a *Dual-PAM-100* system (*Heinz Walz*, Germany) according to Pfündel *et al.* (2008). All algae samples were dark-adapted for 5 min prior to measurements. Minimum fluorescence (F_0) was detected by a measuring light of low intensity. A saturating pulse [10,000 μmol(photon) m^{-2} s^{-1}, duration of 300 ms] was then applied to detect the maximum fluorescence (F_m) after dark-adaptation. The maximal change in P700 signal (P_m) was measured through the application of the saturating pulse after far-red [≈ 5 μmol(photon) m^{-2} s^{-1}] preillumination for 10 s according to the methods of Klughammer and Schreiber (2008). 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 the maximum $P700^+$ signal (P_m') under the actinic light. The slow induction curve was recorded with the routine program. The slow induction curve was recorded for 240 s to achieve the steady state of the photosynthetic apparatus, and then the actinic light was turned off. The data derived after the final saturating pulse was used for analysis of activities of PSI and PSII based on previously determined F_0, F_m, and P_m.

The quantum yields of PSI and PSII were measured by saturating pulses during the process of slow induction curve. Parameters were evaluated automatically according to the methods of Kramer *et al*. (2004) and Klughammer and Schreiber (2008): $Y_{II} = (F_m' - F)/F_m'$, $Y_{NPQ} = F/F_m' - F/F_m$, $Y_{NO} = F/F_m$ (where Y_{II} was the effective photochemical quantum yield of PSII, Y_{NPQ} was regulated energy dissipation, and Y_{NO} was nonregulated 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 was effective photochemical quantum yield of PSI, Y_{ND} was the quantum yield of non-photochemical energy dissipation in reaction centers due to PSI donor-side limitation, Y_{NA} was the quantum yield of nonphotochemical energy dissipation of reaction centers due to PSI acceptor-side limitation).

Calculation of cyclic electron flow (CEF) and linear electron flow (LEF): CEF and LEF were assessed by calculating ratios of Y_{CEF}/Y_I, Y_{CEF}/Y_{II}, and Y_{II}/Y_I. The quantum yield of CEF was calculated as the difference between Y_I and Y_{II}: $Y_{CEF} = Y_I - Y_{II}$ (Miyake *et al.* 2005, Huang *et al.* 2010). Y_{CEF}/Y_I, Y_{II}/Y_I, and Y_{CEF}/Y_{II} indicated 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 (Huang *et al*. 2010, Wang *et al*. 2013).

Measurement of electron transport in PSI and PSII: Electron transport rate (ETR) in PSI and PSII, *i.e.*, ETR_I and ETR_{II}, respectively, were recorded during the measurement of the slow induction curve. They were calculated as $ETR_I = Y_I \times PAR \times 0.84 \times 0.5$, and $ETR_{II} = Y_{II} \times PAR \times 0.84 \times 0.5$ (Maxwell and Johnson 2000, Suzuki *et al.* 2011). The responses of the activities of electron transport in PSI and PSII to increasing PAR from 0 to 1,957 μmol(photon) m^{-2} s^{-1} were recorded as the rapid light curves (RLCs). The following parameters of ETR_I and ETR_{II} in light response reaction were derived from the RLCs according to the exponential function (Platt *et al.* 1980): α, the initial slope of RLC of ETR_I or ETR_{II}, which reflected the quantum yield of PSI or PSII (Saroussi and Beer 2007); ETR_{max}, the maximal electron transport rates in PSI or PSII; I_k, the light saturation of PSI or PSII, which was calculated as ETR_{max}/α. I_k has been found to be related to NPQ (White *et al*. 2011). Photoinhibition detected by RLCs provides the threshold of irradiance a culture can tolerate and indicates at which light intensities photodamage occurs (Schreiber *et al*. 2002, White *et al.* 2011).

Statistical analysis: The experiments were done in triplicate for all treatments. Means and standard deviation were calculated. The statistical significance between control and erythromycin treated samples were calculated in one-way analysis of variance (*ANOVA*) by *SPSS v. 16.0* analyses with posthoc *Fisher*'s least significant difference (LSD) test.

Results

Effects on quantum yield of PSI and PSII: Quantum yield of PSI and PSII were significantly affected after erythromycin exposure (Fig. 1). Data for treatments with 10 and 25 mg L^{-1} erythromycin did not differ significantly in most cases, thus data for 25 mg L^{-1} erythromycin treatment were not shown in Figs. 1*A*,*B*, 2, 3. The Y_I declined when the erythromycin concentration reached from 5 mg L^{-1} to 25 mg L^{-1}. The Y_{ND} markedly increased with rising erythromycin concentration. At 10 mg L^{-1} erythromycin, the Y_I decreased by 8%, while the Y_{ND} increased by 314% (Fig. 1). The Y_{NA} was lowered from 0.181 to 0.083 at 10 mg L^{-1} of erythromycin. The Y_{II} gradually declined when erythromycin concentration rose, meanwhile accompanied with an increase of the Y_{NO}. When erythromycin concentration reached 10 mg L^{-1}, the Y_{II} decreased by 55% while the Y_{NO} increased by 18%. The Y_{NPQ} was kept at zero.

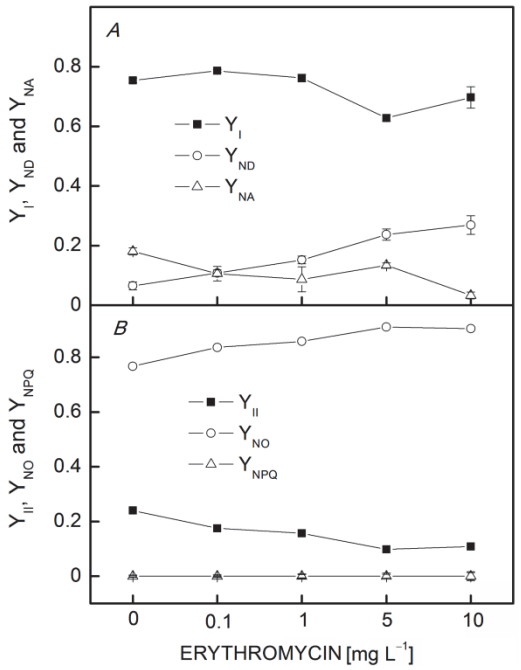

Fig. 1. The quantum yield of PSI (Y_I), nonphotochemical energy dissipation due to donor-side limitation (Y_{ND}), and nonphotochemical energy dissipation due to acceptor-side limitation of PSI (Y_{NA}) (*A*); quantum yield of PSII (Y_{II}), nonregulated energy dissipation (Y_{NO}), and regulated energy dissipation of PSII (Y_{NPQ}) (*B*) of *Microcystis aeruginosa* exposed to various concentrations of erythromycin for 12 h. All data presented were mean values of triplicates. Error bars represented standard deviation.

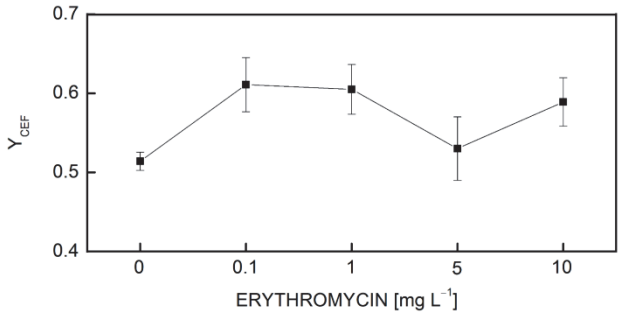

Fig. 2. The quantum yield of cyclic electron flow (Y_{CEF}) of *Microcystis aeruginosa* exposed to various concentrations of erythromycin after 12 h. $n = 3$. Error bars = SD.

Effects on CEF and LEF: The Y_{CEF} of *M. aeruginosa* increased significantly at 0.1–1 mg L^{-1} of erythromycin and decreased but remained higher than that of the control at 5–10 mg L^{-1} of erythromycin (Fig. 2). The ratios of Y_{CEF}/Y_I and Y_{CEF}/Y_{II} significantly increased with rising erythromycin concentration (Fig. 3). The Y_{CEF}/Y_I increased by 24% at 10 mg L^{-1} erythromycin compared with the control. The Y_{CEF}/Y_{II} elevated by 155% in the presence of 10 mg L^{-1} erythromycin in comparison with the control. The Y_{II}/Y_I was significantly reduced by 51% at 10 mg L^{-1} of erythromycin (Fig. 3).

Effects on electron transport of PSI and PSII: The rapid light curves (RLCs) of ETR_I and ETR_{II} of *M. aeruginosa* treated with various concentrations of erythromycin were shown in Fig. 4 and derived parameters from RLCs were shown in Table 1. $ETR_{max(II)}$ and $ETR_{max(I)}$ declined

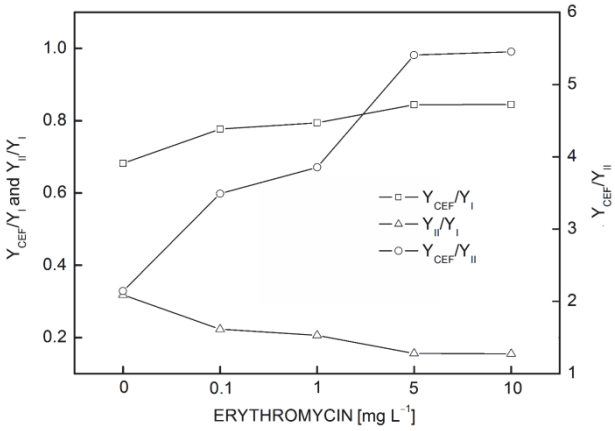

Fig. 3. The ratio of the contribution of cyclic electron flow (CEF) to Y_I (Y_{CEF}/Y_I), the ratio of the quantum yield of cyclic electron flow to linear electron flow (Y_{CEF}/Y_{II}), and the distribution of quantum yield between two photosystems (Y_{II}/Y_I) of *Microcystis aeruginosa* exposed to various concentrations of erythromycin for 12 h. $n = 3$. Error bars = SD.

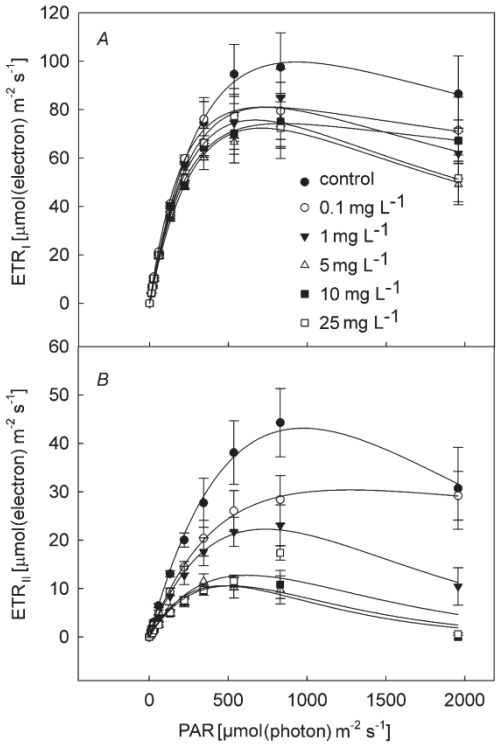

Fig. 4. The rapid light curves of ETR_I and ETR_{II} were recorded by the *Dual-PAM* system during the light response reaction, where PAR increased from 0 to 1,957 µmol(photon) m^{-2} s^{-1}. $n = 3$. Error bars = SD.

Table 1. Parameters (α – the initial slope of RLC of ETR, ETR_{max} – the maximum electron transport rates, I_k – the light saturation) of electron transport in PSII and PSI, which were derived from the rapid light curves (RLCs) of ETR_{II} and ETR_I according to Platt *et al*. (1980). Data represent averages of three RLCs ± SD (n = 3).

	Parameters of RLCs of PSII			Parameters of RLCs of PSI		
Erythromycin [mg L^{-1}]	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	0.09 ± 0.01	43.4 ± 3.1	470.8 ± 25.3	0.376 ± 0.029	100.0 ± 7.3	266.2 ± 20.4
0.1	0.09 ± 0.01	30.6 ± 3.5	346.2 ± 223.8	0.476 ± 0.387	82.7 ± 5.6	173.6 ± 15.8
1	0.05 ± 0.01	22.8 ± 2.8	420.7 ± 41.0	0.399 ± 0.263	80.4 ± 6.6	201.7 ± 14.3
5	0.06 ± 0.01	10.6 ± 1.3	170.4 ± 16.9	0.331 ± 0.022	72.4 ± 5.9	218.6 ± 22.5
10	0.06 ± 0.00	10.6 ± 0.6	190.5 ± 10.8	0.369 ± 0.048	74.3 ± 7.1	201.3 ± 13.1
25	0.06 ± 0.00	12.8 ± 1.1	228.5 ± 19.4	0.374 ± 0.029	75.7 ± 7.8	202.3 ± 10.6

significantly with increasing erythromycin concentration (Table 1). The value of $ETR_{max(II)}$ and $ETR_{max(I)}$ decreased by 71 and 24.3%, respectively, when algal samples were exposed to 25 mg L^{-1} of erythromycin. Compared with the control, $I_{k(II)}$ and $I_{k(I)}$ were markedly reduced with increasing erythromycin concentration. In the presence of 25 mg L^{-1} erythromycin, $I_{k(II)}$ decreased from 470.8 (control) to 228.5 μmol(photon) m^{-2} s^{-1} and $I_{k(I)}$ decreased from 266.2 (control) to 202.3 μmol(photon) m^{-2} s^{-1} (Table 1).

Discussion

In present study, effects of erythromycin were analyzed on activities and electron transport of PSI and PSII, and the energy regulation mechanism between PSI and PSII in *M. aeruginosa*. The decrease of the Y_{II} accompanied by the increase of the Y_{NO} (Fig. 1*B*) reflected the significant inhibitory effect of erythromycin (≥ 5 mg L^{-1}) on photochemical energy utilization of PSII. The Y_{NO} usually reflects the fraction of energy that is passively dissipated in the form of heat and fluorescence mainly due to the closed PSII centers (Klughammer and Schreiber 2008, Pfündel *et al.* 2008, Suzuki *et al.* 2011). It suggests that excessive excitation energy cannot be efficiently dissipated into harmless heat and the PSII energy-regulation mechanism was inhibited at ≥ 5 mg L^{-1} erythromycin (Suzuki *et al.* 2011). Erythromycin may penetrate the cell walls of bacteria and reversibly binds to the 50S subunit of the ribosome, inhibiting RNA-dependent protein synthesis of bacteria (Pestka 1976, Chittum and Champney 1995). Some thylakoid membrane proteins are encoded by chloroplast genes (Liu *et al*. 2011), thus erythromycin may interfere *via* the inhibition of the synthesis of these proteins. Erythromycin can also inhibit the synthesis of the D1 protein (Liu *et al*. 2011), which is important to maintain the stability of Q_B in PSII reaction center (Strasser 1997), and this causes the inefficiency of PSII. The inhibitory effects of erythromycin on photosynthesis might be also correlated with the decrease of Chl *a*. The significant decrease of $ETR_{max(II)}$ after the exposure to erythromycin indicated that the electron transport of PSII was seriously inhibited by erythromycin. A clear, concentration-dependent relationship for erythromycin was obtained, which agreed with González-Pleiter *et al*. (2013).

$ETR_{max(II)}$ and $ETR_{max(I)}$ decreased with the increase of erythromycin concentration, indicating the significant inhibitory effect of erythromycin on electron transport in PSII and PSI. As the cyanobacterium cells were in the logarithmic phase, the lowered $I_{k(II)}$ and $I_{k(I)}$ after the exposure to erythromycin showed that the treatment triggered photoinhibition of PSII and PSI. The threshold of irradiance that cyanobacteria tolerate decreased, and enhanced photodamage, especially to PSII, occurred at lower light intensities (White *et al.* 2011). It was similar to the pea leaves under heavy metal stress (Wodala *et al*. 2012). The significant decline of $I_{k(II)}$ and $ETR_{max(II)}$ reflected the decreased efficiency of PSII to use high illumination under the toxic stress of erythromycin (Wang *et al*. 2013).

The photosynthetic quantum yield and ETR were inhibited less in PSI than in PSII (Fig. 4, Table 1). It was consistent with previous studies that PSI was usually more stable than PSII under stress (Wang *et al.* 2013). Inhibition of PSI activity by ≥ 5 mg L^{-1} erythromycin was associated with the increase of the Y_{ND} and the decrease of the Y_{NA} (Fig. 1*A*), implying that the decrease of quantum yield of PSI occurred mainly due to the limitation of the donor side of PSI. Increase of the Y_{ND} implies that the fraction of oxidized P700 lacking donors from PSII increases (Huang *et al*. 2010), and the inefficiency of PSII functioning is caused by the reduced $ETR_{max(II)}$ 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} (Pfündel *et al.* 2008, Gao *et al*. 2011). The decrease of the Y_{NA} showed that *M. aeruginosa* protected itself against damage by converting excess radiation to nonphotochemical energy dissipation (Klughammer and Schreiber 2008).

When erythromycin concentration increased from

0.1 to 1 mg L^{-1}, the Y_{CEF} increased significantly (Fig. 3). CEF around PSI is essential to dissipate photon energy for preventing damage (Li *et al*. 2006). CEF has also been found to be stimulated under some other stressful conditions (Jin *et al*. 2008, Lu *et al*. 2008, Gao and Wang 2012). The increase of CEF implies the stimulation of the protection mechanism around PSI (Takahashi *et al*. 2009) under lower erythromycin concentration treatment. In other words, CEF can transfer electrons from PSI to PQ and protect PSII by dissipating excessive energy and prevent acceptor-side limitation of PSI (Munekage *et al*. 2002, Takahashi *et al.* 2009, Huang *et al*. 2012). It is suggested that CEF protects PSII by producing a pH gradient and dissipating excess photon energy by inducing NPQ (Miyake *et al.* 2005). However, in the present study, NPQ did not increase, which needs further research. However, the Y_{CEF} gradually decreased at erythromycin concentration $\geq$ 5 mg L^{-1}, implying the protection mechanism was weakened at moderate and high erythromycin concentration. The increase of Y_{CEF}/Y_I and Y_{CEF}/Y_{II} with rising erythromycin concentration (Fig. 3) suggested the contribution of CEF to counteracting the inhibitory effect of erythromycin on PSII and PSI. On the contrary, the Y_{II}/Y_I reduced with the increasing erythromycin concentration indicated the decrease of the contribution of LEF to Y_I. Inhibition of LEF from PSII to PSI could help to alleviate the damage to PSI (Kudoh and Sonoike 2002).

In conclusion, PSII was more sensitive than PSI to acute toxicity of erythromycin. The decrease in the quantum yield of PSII and PSI occurred mainly due to photoinhibition of PSII, associated with the increase in the nonregulated energy dissipation and donor-side limitation of PSI. CEF plays an important role in protecting PSI against the toxicity of erythromycin.

References

Alder, A.C., McArdell, C.S., Golet, E.M. *et al*.: Environmental exposure of antibiotics in Switzerland. – In: Kümmerer, K. (ed.): Pharmaceuticals in the Environment: Sources Fate, Effects and Risks. Pp. 63-64. Springer, Freiburg 2004.

Aristilde, L., Melis, A., Sposito, G.: Inhibition of photosynthesis by a fluoroquinolone antibiotic. – Environ. Sci. Technol. **41**: 1444-1450, 2010.

Belatik, A., Hotchandani, S., Tajmir-Riahi, H.A., *et al*.: Alteration of the structure and function of photosystem I by Pb^{2+}. – J. Photoch. Photobio. B. **123**: 41-47, 2013.

Chittum, H.S., Champney, W.S.: Erythromycin inhibits the assembly of the large ribosomal subunit in growing *Escherichia coli* cells. – Curr. Microbiol. **30**: 273-279, 1995.

Fick, J., Söderström, H., Lindberg, R.H. *et al*.: Contamination of surface, ground, and drinking water from pharmaceutical production. – Environ. Toxicol. Chem. **28**: 2522-2527, 2009.

Gao, S., Wang, G.: The enhancement of cyclic electron flow around photosystem I improves the recovery of severely desiccated *Porphyra yezoensis* (Bangiales, Rhodophyta). – J. Exp. Bot. **63**: 4349-4358, 2012.

Gao, S., Shen, S.D., Wang, G.C. *et al*.: PSI-driven cyclic electron flow allows intertidal Macro-algae *Ulva* sp. (Chlorophyta) to survive in desiccated conditions. – Plant Cell Physiol. **52**: 885-893, 2011.

González-Pleiter, M., Gonzalo, S., Rodea-Palomares, I. e*t al*.: Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic organisms: Implication for environmental risk assessment. – Water Res. **47**: 2050-2064, 2013.

Halling-Sørensen, B.: Algal toxicity of antibacterial agents used in intensive farming. – Chemosphere **40**: 731-739, 2000.

Holten-Lützhøft, H.C., Halling-Sørensen, B., Jørgensen, S.E.: Algal toxicity of antibacterial agents applied in Danish fish farming. – Arch. Environ. Con. Tox. **36**: 1-6, 1999.

Huang, W., Yang, S.J., Zhang, S.B. *et al*.: Cyclic electron flow plays an important role in photoprotection for the resurrection plant *Paraboea rufescens* under drought stress. – Planta **235**: 819-828, 2012.

Huang, W., Zhang, S.B., Cao, K.F.: Stimulation of cyclic electron flow during recovery after chilling-induced photoinhibition of PSII. – Plant Cell Physiol. **51**: 1922-1928, 2010.

Isidori, M., Lavorgna, M., Nardelli, A. *et al*.: Toxic and genotoxic evaluation of six antibiotics on non-target organisms. – Sci. Total Environ. **346**: 87-98, 2005.

Jin, S.H., Wang, D., Zhu, F.Y. *et al*.: Up-regulation of cyclic electron flow and down regulation of linear electron flow in antisense-*rca* mutant rice. – Photosynthetica **46**: 506-510, 2008.

Klughammer, C., Schreiber, U.: Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. – PAM Appl. Notes **1**: 27-35, 2008.

Kolpin, D.W., Furlong, E.T., Meyer, M.T. *et al*.: PharmaceuticalsF hormones, and other organic wastewater contaminants in US streams, 1999 – 2000: a national reconnaissance. – Environ. Sci. Technol. **36**: 1202-1211, 2002.

Kramer, D.M., Johnson, G., Kiirats, O., Edwards, G.E.: New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. – Photosynth. Res. **79**: 209-218, 2004.

Kudoh, H., Sonoike, K.: Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. – Planta **215**: 541-548, 2002.

Kümmerer, K.: Antibiotics in the aquatic environment. A review. Part I. – Chemosphere **75**: 417-434, 2009a.

Kümmerer, K.: Antibiotics in the aquatic environment. A review. Part II. – Chemosphere **75**: 435-441, 2009b.

Li, X.G., Xu, P.L., Zhao, J.P. *et al*.: Ferredoxin-quinone reductase benefits cyclic electron flow around photosystem I in tobacco leaves upon exposure to chilling stress under low irradiance. – Photosynthetica **44**: 349-354, 2006.

Lin, A.Y.C., Tsai, Y.T.: Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities. – Sci. Total Environ. **407**: 3793-3802, 2009.

Liu, B.Y., Nie, X.P., Liu, W.Q. *et al*.: Toxic effects of erythromycin, ciprofloxacin and sulfamethoxazole on photosynthetic apparatus in *Selenastrum capricornutum*. – Ecotox. Environ. Safe. **74**: 1027-1035, 2011.

Lu, K.X., Yang, Y., He, Y., Jiang, D.A.: Induction of cyclic

electron flow around photosystem I and state transition are correlated with salt tolerance in soybean. – Photosynthetica **46**: 10-16, 2008.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Miyake, C., Horiguchi, S., Makino, A. *et al*.: Effects of light intensity on cyclic electron flow around PSI and its relationship to non-photochemical quenching of Chl fluorescence in tobacco leaves. – Plant Cell Physiol. **46**: 1819-1830, 2005.

Munekage, Y., Hojo, M., Meurer, J. *et al*.: *PGR5* is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. – Cell **110**: 361-371, 2002.

Nie, X.P., Liu, B.Y., Yu, H.J. *et al*.: Toxic effects of erythromycin, ciprofloxacin and sulfamethoxazole exposure to the antioxidant system in *Pseudokirchneriella subcapitata.* – Environ. Pollut. **172**: 23-32, 2013.

Pan, X.L., Deng, C.N., Zhang, D.Y. *et al.*: Toxic effects of amoxicillin on the photosystem II of *Synechocystis* sp. characterized by a variety of *in vivo* chlorophyll fluorescence tests. – Aquat. Toxicol. **89**: 207-213, 2008.

Pan, X.L., Zhang, D.Y., Chen, X. *et al*.: Effects of levofloxacin hydrochlordie on photosystem II activity and heterogeneity of *Synechocystis* sp. – Chemosphere **77**: 413-418, 2009.

Park, S.J., Choi, K.H.: Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. – Ecotoxicology **17**: 526-538, 2008.

Pestka, S.: Insights into protein biosynthesis and ribosome function through inhibitiors. – Prog. Nucleic Acid Res. Mol. Biol. **17**: 217-245, 1976.

Pfündel, E., Klughammer, C., Schreiber, U.: Monitoring the effects of reduced PS II antenna size on quantum yields of photosystems I and II using the Dual-PAM-100 measuring system. – PAM Appl. Notes **1**: 21-24, 2008.

Pinckney, J.L., Hagenbuch, I.M., Long, R.A., Lovell, C.R.: Sublethal effects of the antibiotic tylosin on estuarine benthic microalgal communities. – Mar. Pollut. Bull. **68**: 8-12, 2013.

Platt, T., Gallegos, C.L., Harrison, W.G.: Photoinhibition of photosynthesis in natural assemblages of marine phyto-plankton. – J. Mar. Res. **38**: 687-701, 1980.

Richardson, B.J., Lam, P.K.S., Martin, M.: Emerging chemicals of concern: pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China. – Mar. Pollut. Bull. **50**: 913-920, 2005.

Santos, L.H., Araújo, A.N., Fachini, A., *et al*.: Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. – J. Hazard. Mater. **175**: 45-95, 2010.

Sarmah, A.K., Meyer, M.T., Boxall, A.B.A.: A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. – Chemosphere **65**: 725-759, 2006.

Saroussi, S., Beer, S.: Alpha and quantum yield of aquatic plants derived from PAM fluorometry: Uses and misuses. – Aquat. Bot. **86**: 89-92, 2007.

Schreiber, U., Muller, J.F., Haugg, A., Gademann, R.: New type of dual-channel PAM chlorophyll fluorometer for highly sensitive water toxicity biotests. – Photosynth. Res. **74**: 317-330, 2002.

Scribner, E.A., Dietze, J.E., Meyer, M.T., Kolpin, D.W.: Occurrence of antibiotics in water from fish hatcheries [abs.]. – In: 21st Annual Water and Future of Kansas Conference. Pp. 68. Water Resources Research Institute, New York 2004.

Singh, R., Dubey, G., Singh, V.P. *et al*.: High light intensity augments mercury toxicity in cyanobacterium *Nostoc muscorum*. – Biol. Trace Elem. Res. **149**: 262-272, 2012.

Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G.: Purification and properties of unicellular blue-green algae (order *Chroococcales*). – Bacteriol. Rev. **35**: 171-205, 1971.

Stoichev, T., Baptista, M.S., Basto, M.C.P. *et al*.: Effects of minocycline and its degradation products on the growth of *Microcystis aeruginosa.* – Ecotox. Environ. Safe. **74**: 219-224, 2011.

Strasser, B.J.: Donor side capacity of photosystem II probed by chlorophyll a fluorescence transients. – Photosynth. Res. **52**: 147-155, 1997.

Suzuki, K., Ohmori, Y., Ratel, E.: High root temperature blocks both linear and cyclic electron transport in the dark during chilling of the leaves of rice seedlings. – Plant Cell Physiol. **52**: 1697-1707, 2011.

Takahashi, S., Milward, S.E., Fan, D.Y. *et al*.: How does cyclic electron flow alleviate photoinhibition in *Arabidopsis*? – Plant Physiol. **149**: 1560-1567, 2009.

Wang, S.Z., Chen, F.L., Mu, S.Y. *et al.*: Simutaneous analysis of photosystem responses of *Microcystis aeruginoga* under chromium stress. – Ecotox. Environ. Safe. **88**: 163-168, 2013.

Wang, S.Z., Zhang, D.Y., Pan, X.L.: Effects of arsenic on growth and photosystem II (PSII) activity of *Microcystis aeruginosa*. – Ecotox. Environ. Safe. **84**: 104-111, 2012.

White, S., Anandraj, A., Bux, F.: PAM fluorometry as a tool to assess microalgal nutrient stress and monitor cellular neutral lipids. – Bioresource Technol. **102**: 1675-1682, 2011.

Wise, R.: Antimicrobial resistance: priorities for action. – J. Antimicrob. Chemoth. **49**: 585-586, 2002.

Wodala, B., Eitel, G., Gyula, T.N. *et al*.: Monitoring moderate Cu and Cd toxicity by chlorophyll fluorescence and P_{700} absorbance in pea leaves. – Photosynthetica **50**: 380-386, 2012.

Yang, W.W., Tang, Z.P., Zhou, F.Q. *et al*.: Toxicity studies of tetracycline on *Microcystis aeruginosa* and *Selenasrum capricornutum*. – Environ.Toxicol. Phar. **35**: 320-324, 2013.

Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R..: Presence of therapeutic drugs in the environment. – Lancet **355**: 1789-1790, 2000.