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Elucidating the toxicity targets of β-ionone on photosynthetic system of *Microcystis aeruginosa* NIES-843 (Cyanobacteria)

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ABSTRACT

In order to explore the potential targets of toxicity of β-ionone on the photosynthetic system of *Microcystis aeruginosa*, the polyphasic rise in chlorophyll *a* (Chl *a*) fluorescence transient and transcript expression for key genes in photosystem II (PSII) of *M. aeruginosa* NIES-843 were studied. The EC_{50} value of β-ionone on *M. aeruginosa* NIES-843 was found to be 21.23 ± 1.87 mg/L. It was shown that β-Ionone stress can lead to a decrease in pigment content of *M. aeruginosa* NIES-843 cells, and that carotenoids were more sensitive to β-ionone stress than Chl *a*. The normalized Chl *a* fluorescence transients were slightly decreased at 6.67 and 10 mg/L β-ionone, but significantly increased at 15, 22.5 and 33.75 mg/L. There was no significant variation on transcript expression of *psbA* and *psbO* at a concentration of 6.67 mg/L β-ionone, but they were down-regulated at 22.5 mg/L. Ultrastructural examination by transmission electron microscopy indicated that the thylakoids were distorted, and the thylakoid membrane stacks began to collapse when *M. aeruginosa* NIES-843 was exposed to β-ionone at a concentration of 22.5 and 33.75 mg/L. Our results indicate that the reaction centre of PS II and the electron transport at the acceptor side of PS II are the targets responsible for the toxicity of β-ionone on the PS II of *M. aeruginosa* NIES-843.

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1. Introduction

In recent years, cyanobacteria are often found to dominate in eutrophic waters worldwide. *Microcystis* is the most important genus responsible for the formation of water blooms and production of microcystins (Azevedo et al., 2002). Microcystins can inhibit the eukaryotic protein phosphatases PP1 and PP2A, and cause liver cancer and tumors in humans and wild animals (Carmichael, 1995). Therefore, the control of harmful *M. aeruginosa* is crucial for maintenance of safe water supplies. Some aquatic macrophytes are known to inhibit the growth of water bloom forming cyanobacteria (Nakai et al., 2000; Gross, 2003; Mulderij et al., 2007). Many substances excreted by aquatic macrophytes such as tellimagrandin II (Gross et al., 1996), pyrogallic acid (Nakai et al., 2000), and ethyl-2-methylacetoacetate (Li and Hu, 2005), have algicidal activities. Recently, volatile organic compounds were reported to have lytic effects on *Microcystis* (Ozaki et al., 2008). β-Ionone is a volatile terpenoid compound which has been found to be widely distributed in algae and higher plants. The biosynthesis of β-ionone is derived from β-carotene being cleaved through carotenoid cleavage of dioxygenases (Simkin et al., 2004). Previous work indicated that β-ionone has antimicrobial activity on some pathogenic microorganisms of plants (Schiltz, 1974; Mikhlin et al., 1983; Utama et al., 2002). High concentration of β-ionone was also found in the volatile oils extracted from the aquatic macrophyte *Elodea nuttallii* (Wang, 2009). This aquatic macrophyte was reported to have allelopathic effects on *M. aeruginosa* (Wu et al., 2009). High concentration of β-ionone was also found in cultures of the filamentous cyanobacterium *Tychonema* which also had an allelopathic effect on *M. aeruginosa* (unpublished data). These phenomena indicate that β-ionone may be an allelopathic substance contributing to the inhibitory effects of *E. nuttallii* on *M. aeruginosa*. More recently, β-ionone was reported to cause cellular lysis and a decrease of Chl *a* content on *Microcystis* (Harada et al., 2009). It is known that cellular lysis and a decrease of Chl *a* are common phenomena of algal cell death, but it is not clear what the inhibiting targets are and what the inhibiting mechanism of β-ionone on *Microcystis* is. Moreover, only one relative high concentration of β-ionone (1248 mg/L) was investigated, and such a high concentration of β-ionone would rarely appear in natural waters. The photosynthetic process is a universal feature of algae, and it is sensitive to environmental changes

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(Ma et al., 2006). Chl *a* fluorescence can indicate the photosynthetic efficiency and provide information on the relationship between structure and function of PS II (Christen et al., 2007), In addition the Chl *a* fluorescence transients for dark-adapted photosynthetic organisms, under the illumination of actinic light, has been widely used in detection and evaluation of various stresses on plant photosystem (Pinior et al., 2005; Strauss et al., 2006; Christen et al., 2007) and that of cyanobacteria (Lu and Vonshak, 1999; Bueno et al., 2004; Zhao et al., 2008). Recently, gene expression analysis has also been used to evaluate environmental stress on algae (De Jong et al., 2006; Operaña et al., 2007). In order to explore the potential targets for toxicity from β-ionone on photosynthetic systems of water bloom forming *M. aeruginosa*, Chl *a* fluorescence transients and transcript expressions of key genes related to the photosynthetic system of *M. aeruginosa* under β-ionone stress, were studied.

2. Materials and methods

2.1. Cyanobacterial strain, culture conditions and chemicals

M. aeruginosa NIES-843 was kindly provided by the National Institute of Environmental Science, Japan. It was grown in CT liquid medium (pH 8.2) (Ichimura, 1979), under a 12:12 LD cycle with a light intensity of 30 μmol photons/(s m^2) provided by cool white fluorescent tubes at 25 ± 1 °C. β-Ionone (≥97%) was purchased from the Sigma–Aldrich Chemical Company (Sigma, St. Louis, MO, USA).

2.2. Inhibitory effects of β-ionone on the growth of M. aeruginosa NIES-843

Experiments were carried out in 250 mL conical flasks containing 94.9 mL CT liquid medium, and 100 μL of β-ionone solutions in dimethyl sulfoxide (DMSO). Prior to these experiments, we found that 0.2% (v/v) DMSO had no obvious effect on the growth and photosynthetic processes of *M. aeruginosa* NIES-843. Five milliliters of exponential-phase cultures of *M. aeruginosa* NIES-843 (in CT medium) were added to the conical flasks in order to reach a final volume of 100 mL. The final β-ionone concentrations were set as 0, 6.67, 10, 15, 22.5, 33.75 mg/L, respectively, and the initial cellular concentrations of *M. aeruginosa* NIES-843 were 6.5×10^5 cells/mL. Each treatment was replicated three times. All treatments were cultured under the same conditions as mentioned above. The increase in *M. aeruginosa* cell number was calculated after 48 h using a hemocytometer.

2.3. Determination of photosynthetic pigments

Chl *a* and carotenoid content were determined according to the method described by Wang (2000). Cultures were sampled 48 h after inoculation to measure content of photosynthetic pigments. Chl *a* and carotenoids were extracted with 90% ethanol, and the whole procedure was carried out in the dark at 4 °C. Absorption spectra of the pigment extracts were recorded at 700–350 nm using a Simadzu UV-1800 spectrophotometer (Simadzu, Kyoto, Japan).

2.4. Measurement of polyphasic Chl a fluorescence transients

The polyphasic rise in Chl *a* fluorescence transients was measured by a Handy-Plant Efficiency Analyser (Handy-PEA, Hansatech, King's Lynn, Norfolk, UK) with an actinic light of 3000 μmol quanta /(m^2 s). All samples were dark-adapted for 15 min before measurements. Parameters involved in changes of Chl *a* fluorescence induction curves were calculated as follows (for details, see Christen et al., 2007):

$$\frac{dV}{dt_0} = V_K = \frac{F_{300\,\mu s} - F_0}{F_M - F_0}$$

$$V_J = \frac{F_{2\,ms} - F_0}{F_M - F_0}$$

$$\varphi P_0 = 1 - \frac{F_0}{F_M}$$

$$\varphi D_0 = \frac{F_0}{F_M}$$

$$\psi_0 = 1 - V_J$$

$$\varphi E_0 = \left(1 - \frac{F_0}{F_M}\right)(1 - V_J)$$

Table 1
Primers used for Real-Time PCR.

Gene name	Forward primer (5′–3′)	Reverse primer (5′–3′)
16S rrn	GGACGGGTGAGTAACGCGTA[a]	CCCATTGCGGAAAATTCCCC[b]
psbA[c]	GGTCAAGARGAAGAAACCTACAAT	GTTG AAACCGTTGAGGTTGAA
psbO	GTGTCGGAAGGCTGCTCA	CCAAAGGTCGGGGTGTG

[a] Primer modified on the basis of the reference reported by Urbach et al. (1992).
[b] Primer sequence based on the report from Nübel et al. (1997).
[c] Primer sequences for *psbA* based on the report from Shao et al. (2009).

2.5. Determination of the transcription of psbA and psbO

psbA encodes D1 protein of PS II, and *psbO* encodes 33 kD manganese-binding protein of PS II. The transcript expressions for *psbA* and *psbO* in *M. aeruginosa* NIES-843, under β-ionone stress, at concentrations of 6.67 and 22.5 mg/L were determined by Real-Time PCR 48 h after inoculation. Total RNA extraction and reverse transcription were performed according to the method described by Shao et al. (2009). The amplification reactions were performed using a MyiQ™ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) under the following conditions: One cycle at 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 59 °C for 30 s, 72 °C for 15 s. Primers used in this study are listed in Table 1. The expression data from Real-Time PCR were evaluated using C_t values. The16S rRNA gene was used as housekeeping gene to normalize the transcript expression levels for the target genes (Bustin, 2000). The induction ratio was calculated using $2^{-\Delta\Delta C_t}$ where $\Delta\Delta C_t = (C_{t,\text{ target gene}} - C_{t,\text{ 16S rrn}})_{\text{stress}} - (C_{t,\text{ target gene}} - C_{t,\text{ 16S rrn}})_{\text{control}}$.

2.6. Transmission electron microscopic observations

M. aeruginosa NIES-843 was inoculated into CT medium containing 0, 22.5 and 33.75 mg/L β-ionone. Cells were harvested, after 48 h exposure to β-ionone, and fixed at room temperature for 2 h in 2.5% glutaraldehyde buffered with phosphate buffer (pH 7.0). These fixed samples were post-fixed in 1% osmium tetraoxide, dehydrated and embedded in Spurr's resin. Sectioning and staining was as described by Ozaki et al. (2009). The stained samples were then examined using a transmission electron microscope (TEM, H-7000FA, Hitachi, Japan).

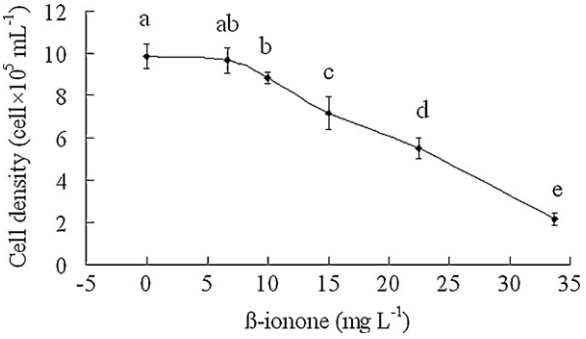

Fig. 1. Effects of β-ionone on the growth of *M. aeruginosa* NIES-843. Average values ± standard deviation ($n = 3$). The symbols 'a', 'b', 'c', 'd' and 'e' represent significance at $P < 0.05$.

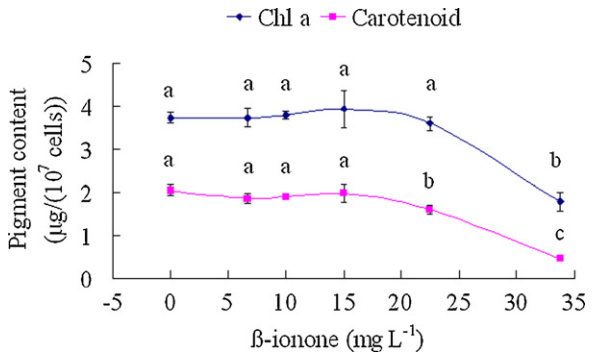

Fig. 2. Effects of β-ionone on the pigment content of *M. aeruginosa* NIES-843. Average values ± standard deviation ($n = 3$). The symbols 'a', 'b' and 'c' represent significance at $P < 0.05$.

2.7. Determination of β-ionone

Harvested cells of *M. aeruginosa* NIES-843, after 48 h exposure to β-ionone, were analyzed for intracellular β-ionone content using Headspace Solid Phase Micro-Extraction (HS-SPME) coupled with GC according to Li et al. (2007), with some modifications. The SPME fiber (PDMS/DVB, 65 μm, Supelco, Bellefonte, PA, USA) was used for the absorption of β-ionone, and a gas chromatography (GC)-2014C (Shimadzu, Kyoto, Japan) equipped with a FID detector was utilized for the β-ionone determination. The separation of extracted compounds was conducted on a capillary column (TC series, WondaCap 5, 0.25 mm × 30m × 0.25 μm, Shimadzu, Kyoto, Japan). The injector and FID detector temperature was set to 250 °C and 270 °C respectively. The oven temperature program was: initial temperature of 60 °C for 2 min; increasing to 200 °C at a rate of 5 °C/min and then held at this temperature for 2 min; followed by an increase to 250 °C at 20 °C/min. This temperature was held for 2 min. High purity N_2 (≥99.99%) was used as the carrier gas under a pressure of 150 kpa. The β-ionone standard solution was used to verify the analysis results for the GC and determine β-ionone content in the cells. Peaks corresponding to the β-ionone standard were also confirmed by GC–MS (HP6890GC-5973MSD, Hewlett-Packard, Wilmington, DE, USA).

2.8. Statistics

Significant differences were determined by one-way ANOVA followed by LSD post hoc test using analysis software (SPSS, version 13.0, SPSS Inc., Chicago, IL, USA). Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Growth characteristics

The effects of β-ionone on the growth of *M. aeruginosa* NIES-843 are shown in Fig. 1. Compared with the controls, there was no significant difference on the growth of *M. aeruginosa* NIES-843 at concentrations less than 6.67 mg/L. The inhibitory effect on growth of *M. aeruginosa* NIES-843 was observed at a concentration of 10 mg/L, and an aggravated inhibition was shown along with the increase in β-ionone concentration. Regression analysis indicated that the EC_{50} of β-ionone on the growth of *M. aeruginosa* NIES-843 was 21.23 ± 1.87 mg/L.

3.2. Photosynthetic pigment content

Changes in Chl *a* and carotenoid content in response to β-ionone stress are shown in Fig. 2. Compared with the controls, no significant differences of Chl *a* content and carotenoid content were found at β-ionone concentrations of less than 22.5 mg/L and less than 15 mg/L, respectively. Chl *a* and carotenoid contents were all significantly lower than those of controls at β-ionone concentrations of 33.75 mg/L and 22.5 mg/L, respectively. Absorption spectra of the pigment extracts at 700–350 nm are shown in Fig. 3. These results indicate that β-ionone stress did not change the constitution and relative rate of synthesis/breakdown of carotenoid ingredients in *M. aeruginosa* NIES-843, although cellular carotenoid content decreased.

3.3. Polyphasic Chl a fluorescence transients

Typical phases for O-J-I-P are presented in the polyphasic Chl *a* fluorescence transients for *M. aeruginosa* NIES-843 (Fig. 4). The normalized fluorescence of Chl *a* fluorescence transients became slightly lower at treatments of 6.67 and 10 mg/L β-ionone, but are significantly higher at 15, 22.5 and 33.75 mg/L β-ionone stress than in controls. Phase I and phase P became obscure from the curves of Chl *a* fluorescence transients when *M. aeruginosa* NIES-843 was grown under the stress of 22.5 mg/L β-ionone, and phase I and phase P all disappeared at 33.75 mg/L β-ionone concentration. Seven parameters including the minimal fluorescence intensity (F_0), slope at the origin of the normalized fluorescence rise (dV/dt_0), $[1-(V_K/V_J)]_{\text{treated sample}}/[1-(V_K/V_J)]_{\text{control}}$, and the energy flux ratios (φD_0, φP_0, φE_0, ψ_0) that deviated from Chl *a* fluorescence induction curves are shown in Figs. 5–8. Compared with controls, the treated *Microcystis* cells had a significantly lower F_0 values at concentrations of 6.67 and 10 mg/L, but had significantly higher F_0 values at concentrations of 15, 22.5 and 33.75 mg/L.

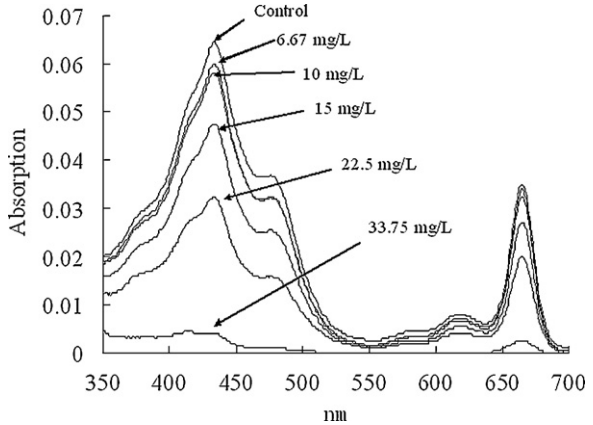

Fig. 3. Absorption spectra of pigment extracts of *M. aeruginosa* NIES-843 under the β-ionone stress.

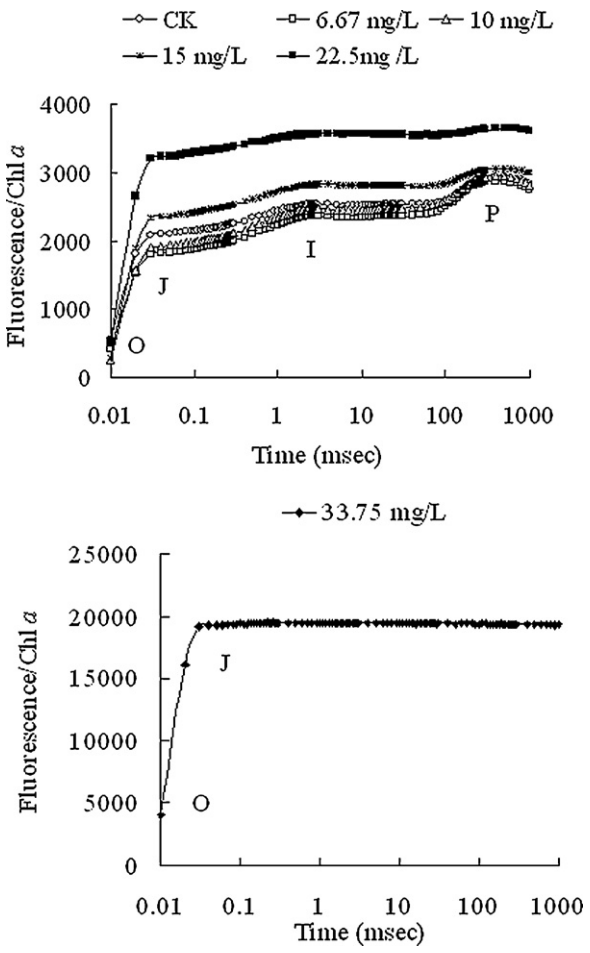

Fig. 4. Chlorophyll *a* fluorescence transients of *M. aeruginosa* NIES 843 under the stress of β-ionone.

The value of dV/dt_0 did not change significantly at concentrations less than 10 mg/L, but it was significantly higher than that of controls at β-ionone concentrations of 15, 22.5 and 33.75 mg/L. The relative value of $[1-(V_K/V_J)]$ was significantly higher than that of the controls at β-ionone concentration of 6.67 mg/L, but was significantly lower than that of the controls at β-ionone concentrations of 15, 22.5 and 33.75 mg/L. φP_0 represents the maximum quantum yield of primary photochemistry and φE_0 represents a

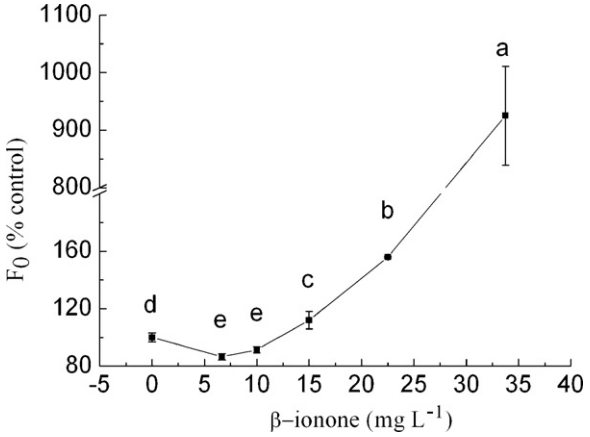

Fig. 5. Effects of β-ionone on the minimal fluorescence intensity (F_0) of *M. aeruginosa* NIES-843 chlorophyll. Average values ± standard deviation ($n = 3$). The symbols 'a', 'b', 'c', 'd' and 'e' represent significance at $P < 0.05$.

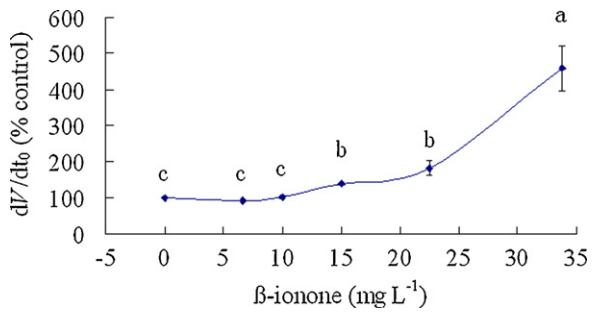

Fig. 6. Effects of β-ionone on dV/dt_0 of chlorophyll fluorescence of *M. aeruginosa* NIES-843. Average values ± standard deviation ($n = 3$). The symbols 'a', 'b' and 'c' represent significance at $P < 0.05$.

probability that an absorbed photon moves an electron further than Q_A^-. φP_0 and φE_0 showed the same variation trends under the stress of β-ionone. Both φP_0 and φE_0 were stimulated at concentrations of 6.67 and 10 mg/L, but φP_0 was depressed by β-ionone at concentrations of 22.5 and 33.75 mg/L, and φE_0 was depressed by β-ionone at concentrations of 15, 22.5 mg/L, and the value of φE_0 dropped to lower than detectable limits of the Handy-PEA at the concentration of 33.75 mg/L. In contrast, maximum quantum yield for non photochemical deexcitation (φD_0) was lower than that of the controls at β-ionone concentrations of 6.67 and 10 mg/L, but significantly higher than that of the controls at β-ionone concentrations of 22.5 and 33.75 mg/L. ψ_0 represents the probability that a trapped exciton moves an electron further than Q_A^-. Compared with the controls, there was no significant difference on the ψ_0 at β-ionone concentrations of 6.67 and 10 mg/L, but it was significantly inhibited at β-ionone concentrations of 15 and 22.5, and the value of ψ_0 dropped to a level lower than detectable limits of the Handy-PEA at β-ionone concentration of 33.75 mg/L.

3.4. Ultrastructure of *M. aeruginosa* NIES-843

Ultrastructural examination on *M. aeruginosa* NIES-843 through TEM indicated that no damage to cell wall and cell membrane was induced by β-ionone (Fig. 9), but the thylakoids were shown to be distorted, and the thylakoid membrane stacks began to collapse when *M. aeruginosa* NIES-843 was exposed to β-ionone at a concentration of 22.5 mg/L. These changes increased at 33.75 mg/L, and the cellular structure disappeared.

3.5. Transcript expression of *psbA* and *psbO* genes

The transcript expression of *psbA* and *psbO* for *M. aeruginosa* NIES-843 exhibited the same trends under stress from β-ionone (Fig. 10). There was no significant difference in the expressions

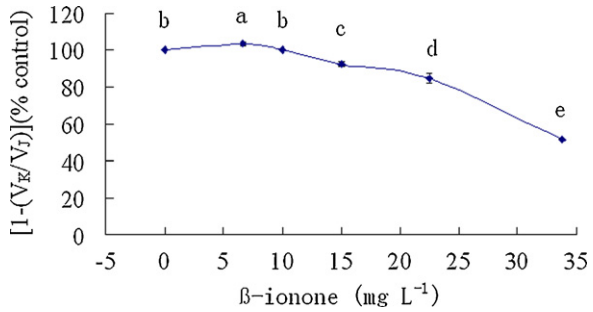

Fig. 7. Effect of β-ionone stress on relative value of $[1-(V_K/V_J)]$ of chlorophyll fluorescence of *M. aeruginosa* NIES-843. Average values ± standard deviation ($n = 3$). The symbols 'a', 'b', 'c', 'd' and 'e' represent significance at $P < 0.05$.

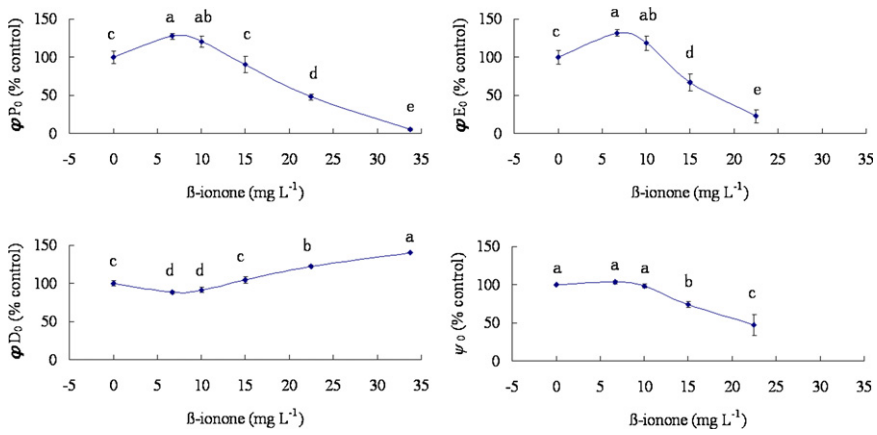

Fig. 8. Effects of β-ionone on the parameters of energy flux ratios (φP_0, φE_0, φD_0, ψ_0) deviated from Chl *a* fluorescence induction curves. Average values ± standard deviation ($n = 3$). The symbols 'a', 'b', 'c', 'd' and 'e' represent significance at $P < 0.05$.

of *psbA* and *psbO* at β-ionone concentration of 6.67 mg/L, but the expressions of *psbA* and *psbO* were significantly depressed at a concentration of 22.5 mg/L β-ionone.

3.6. Intracellular β-ionone content

The β-ionone contents in cells of *M. aeruginosa* NIES-843 are shown in Fig. 11. β-Ionone was detected as 1.18 ng/g WT (wet weight) in the cells of controls. The β-ionone content in the cells increased significantly when *M. aeruginosa* NIES-843 was exposed to β-ionone, and the intracellular β-ionone content reached 1.49 mg/g WT at 33.75 mg/L.

4. Discussion

β-Ionone is a volatile terpenoid compound widely distributed in algae and plants. Some studies have found that β-ionone exhibits inhibitory effects to some pathogenic microorganism of plants (Schiltz, 1974; Mikhlin et al., 1983; Utama et al., 2002). These results indicate that β-ionone may act as an antimicrobial sub-

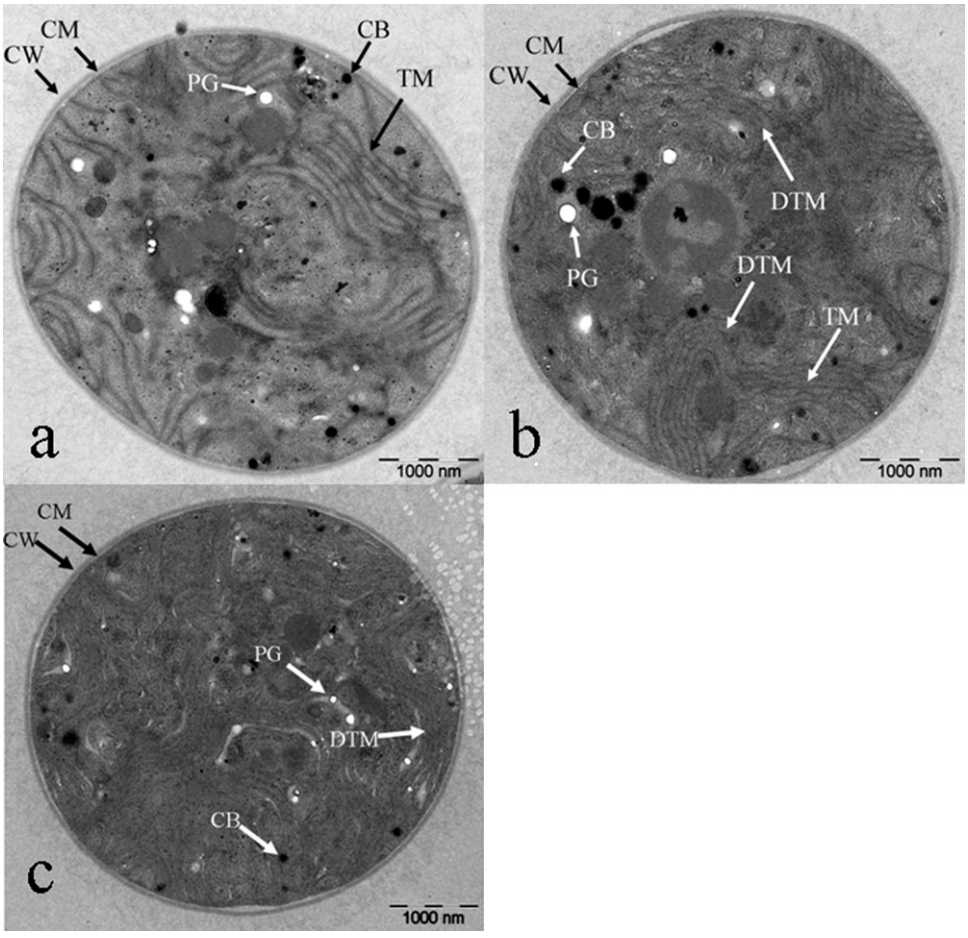

Fig. 9. Effects of β-ionone on the ultrastructure of *M. aeruginosa* NIES-843. a: Control, b: 22.5 mg/L, c: 33.75 mg/L. CW: cell wall; CM: cell membrane; CB: carboxysome; PG: polyphosphate granules; TM: thylakoid membrane; DTM: distorted thylakoid membrane.

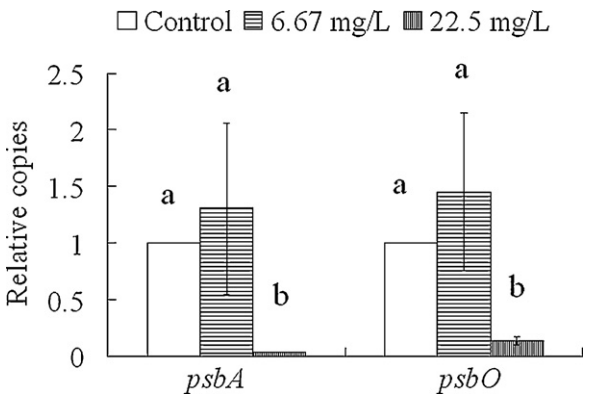

Fig. 10. Effects of β-ionone on the transcript expression of *psbA* and *psbO* genes of *M. aeruginosa* NIES-843. The symbols 'a' and 'b' represent significance at $P < 0.05$.

stance. Harada et al. (2009) reported that β-ionone could cause the lysis of *M. aeruginosa*, but only one extremely high concentration of β-ionone (1248 mg/L) was investigated in their paper, and we could not deduce the inhibitory characters of β-ionone on *Microcystis* from their report. Our results demonstrated that the EC_{50} of β-ionone on the growth of *M. aeruginosa* NIES-843 was 21.23 ± 1.87 mg/L. Comparing the EC_{50} values among β-ionone and other reported allelochemicals, the inhibitory effect of β-ionone was shown to be weaker than that of some stronger allelochemicals, such as pyrogallol (Nakai et al., 2000), nonanoic acid (Nakai et al., 2005) and ethyl-2-methylacetoacetate (Li and Hu, 2005), but it is stronger than that of hexadecanoic acid and 4-hydroxybenzoic (Wang et al., 2008). β-ionone is a lipophilic chemical, and the lipophilic chemicals tend to be absorbed at the surface of algae, and to easily pass through membranes. This may be the reason that the β-ionone content found in the cells of *M. aeruginosa* NIES-843 was far higher than that in the medium. Though the concentration of β-ionone in cultures of *E. nuttallii* could not reach the high levels which showed an inhibitory effect on *Microcystis*, the highly bioaccumulative ability of β-ionone in the cells indicated that intracellular β-ionone content may reach an effective level if the cells of *M. aeruginosa* NIES-843 exposed to β-ionone for a long time even at a relative low concentration. Furthermore, it is also known that synergistic effects widely exist among allolechemicals (Wang et al., 2008; Nakai et al., 2000), and the biotoxicity of β-ionone may be enhanced by some other allolechemicals. Therefore, we speculate that β-ionone may be one of several allelochemicals contributing to the inhibitory effect of *E. nuttallii* on *Microcystis*.

Our results indicate that β-ionone stress could lead to a decrease in pigment content in cells of *M. aeruginosa* NIES-843. As shown in Fig. 2, the carotenoid content of *M. aeruginosa* NIES-843 was

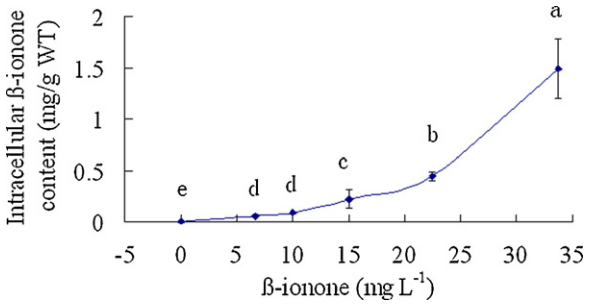

Fig. 11. Intracellular β-ionone contents of *M. aeruginosa* NIES-843 after exposed to β-ionone. Average values ± standard deviation ($n = 3$). The symbols 'a', 'b', 'c', 'd' and 'e' represent significance at $P < 0.05$.

significantly lower than that of the controls at concentrations of 22.5 and 33.75 mg/L β-ionone, but Chl *a* content was shown to be significantly lower only at the 33.75 mg/L β-ionone. This result indicates that carotenoid exhibited more sensitivity to β-ionone stress than Chl *a*. As showed in Fig. 3, β-ionone stress did not change the constitution and relative rate of carotenoid ingredients in *M. aeruginosa* NIES-843, although cellular carotenoid content decreased. The biosynthesis of β-ionone is derived from β-carotene. Trace β-ionone was also detected in the volatile compounds from the *M. aeruginosa* NIES-843 cultures. These results implied that the feedback inhibitory targets for the biosynthesis of β-ionone were the enzymes located upstream of the carotenoid biosynthetic pathway.

Chl *a* fluorescence transients provided considerable information about the photosynthetic apparatus. F_0 corresponds to the minimal fluorescence yield of photochemistry when all molecules of Q_A are in the oxidized state. Higher yield of F_0 means lower efficiency of photochemistry. Destruction of the PS II is always accompanied with an increase of F_0 (Huang et al., 1997; Mallakin et al., 2002; Kummerová et al., 2006). The increase of F_0 shown in the present study indicated that the stress of β-ionone could cause damage to the PS II in *M. aeruginosa* NIES-843, and the decreases of φP_0 and φE_0 also proved this. As mentioned above, ψ_0 represents the probability in which a trapped exciton moves an electron further than Q_A^-, reflecting the state of electron transport at the acceptor side of PS II (Lu and Vonshak, 1999). The stress of β-ionone could lead to a decrease of ψ_0 of *M. aeruginosa* NIES-843, suggesting that electron transport at the acceptor side of PS II is an inhibiting target of toxicity of β-ionone on *M. aeruginosa* NIES-843. As for the polyphasic Chl *a* fluorescence transients, Phase O corresponds to the situation when all molecules of Q_A are in the oxidized state, while Q_A was reduced to Q_A^- in the transition from phase O to J, and Q_B pool was reduced when phase J transformed to phase P (Strasser et al., 1995). Phase I and phase P became obscure when *M. aeruginosa* NIES-843 was exposed to β-ionone at a concentration of 22.5 mg/L, and phase I and phase P disappeared from Chl *a* fluorescence induction curves at a concentration of 33.75 mg/L β-ionone. This result indicated that β-ionone stress could cause disassociation of Q_B from thylakoids, and reduced the Q_B pools. dV/dt_0 represents the relative rate of Q_A reduction (Christen et al., 2007). The relative rate of Q_A reduction increased when *M. aeruginosa* NIES-843 under the stress of β-ionone at concentrations more than 15 mg/L (Fig. 6). As mentioned above, Q_A is the primary electron acceptor of photosystem II, and it can accept an electron from excited Chl *a*. Q_B is the secondary electron acceptor of photosystem II, and it accepts the electrons transported from Q_A. It is speculated that the increase in the relative rate of Q_A reduction was caused by block of the electron moving further than Q_A^- induced by β-ionone, and the trapped electron increased the reduction rate of Q_A. This result also indirectly supported the above suggestion that Q_B in the thylakoid membranes is a toxicity target of β-ionone on *M. aeruginosa* NIES-843. $[1-(V_K/V_J)]_{\text{treated sample}}/[1-(V_K/V_J)]_{\text{control}}$ can reflect the state of the oxygen evolution complex for PS II (Appenroth et al., 2001). The values $[1-(V_K/V_J)]_{\text{treated sample}}/[1-(V_K/V_J)]_{\text{control}}$ were much lower than 1 when the concentrations of β-ionone were higher than 15 mg/L (Fig. 7), indicating that the oxygen evolution complex of PS II is another target of toxicity for β-ionone on *M. aeruginosa* NIES-843.

PsbA and PsbO are two important proteins in the oxygen evolution complex for PS II. They are also sensitive to environmental stress (Gong et al., 2008; Zhao et al., 2008), and the damaged D1 protein in PS II is continuously replaced by a newly synthesized precursor to the D1 protein encoded by *psbA* (Nishiyama et al., 2005). The down regulated transcript expressions of *psbA* and *psbO* for *M. aeruginosa* NIES-843 under β-ionone stress could cause the insufficiency of PsbA and PsbO in the PS II. Such a result may be the

reason for the toxicity of β-ionone on the reaction centre of PS II of *M. aeruginosa* NIES-843.

Chlorophyll *a*-protein complexes are embedded in the thylakoid membranes of cyanobacteria. Ultrastructural examination by TEM demonstrated that the damage to the thylakoid membrane occurred at β-ionone concentrations of 22.5 and 33.75 mg/L. This result indicated that 22.5 mg/L β-ionone could cause the photosynthetic complex to disaggregate. Results based on pigment determination demonstrated that there was no obvious decrease on the cellular Chl *a* content of *M. aeruginosa* NIES-843, when the cells were exposed to 22.5 mg/L β-ionone concentration. Combining the results from ultrastructural change and pigment levels, it is suggested that 22.5 mg/L β-ionone stress could cause an increase in dissociated Chl *a* for the thylakoid membranes of *M. aeruginosa* NIES-843. The dissociated Chl *a* can be excited but loses the ability to transport electrons through the electron transport chain. One important way for quenching the excited dissociated Chl *a* is the emission of fluorescence, accounting for an increase of the normalized fluorescence for Chl *a* under the stress of β-ionone (Fig. 4).

Many environmental toxins were reported to enhance the photosynthetic activity of cyanobacteria and plants at low concentrations but inhibit the photosynthetic activity at high concentrations (Chen et al., 2007; Zhou et al., 2006; Nyitrai et al., 2009). The changes of φP_0 and φE_0 under β-ionone stress also exhibit this trend. Low concentration of β-ionone ($\leq$10 mg/L) could increase photochemical efficiency, but high concentration of β-ionone ($\geq$15 mg/L) could inhibit photochemical efficiency. Photosynthesis is the process of converting light energy to chemical energy. Intracellular ATP, the universal chemical energy molecule in cells, is required for the adaptation of cyanobacterial cells and plants to stress (Howitt et al., 2001; Zhou et al., 2006). More energy being required for the adaptation of *M. aeruginosa* NIES-843 to β-ionone stress may be the reason for the increase of photochemical efficiency under the stress of low concentrations of β-ionone.

5. Conclusion

The EC_{50} of β-ionone on the growth of *M. aeruginosa* NIES-843 was shown to be 21.23 ± 1.87 mg/L. Results based on transcript expression of genes, polyphasic Chl *a* fluorescence transients and ultrastructural examinations through TEM indicated that the reaction centre of PS II and electron transport at the acceptor side of PS II are the targets responsible for the toxicity of β-ionone on the PS II of *M. aeruginosa* NIES-843.

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