Int. J. Environ. Sci. Technol. (2014) 11:469–476
DOI 10.1007/s13762-013-0205-4

ORIGINAL PAPER

Interactions between algicidal bacteria and the cyanobacterium *Microcystis aeruginosa*: lytic characteristics and physiological responses in the cyanobacteria

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Received: 31 January 2012 / Revised: 24 December 2012 / Accepted: 2 February 2013 / Published online: 28 February 2013

Abstract Application of algicidal bacteria is a promising and environmentally friendly way to control cyanobacterial blooms. Lytic effects of the algicidal bacteria on *Microcystis aeruginosa* have been observed, but the interactions between algicidal bacteria and the cyanobacteria are still elusive. An algicidal bacterium *Bacillus* sp. B50 isolated from Lake Donghu showed a highly lytic efficiency on *M. aeruginosa* NIES-843 through heat-resistant extracellular substances from strain B50. The cell density of strain B50 could be maintained at high levels during the lytic process in bacteria–*Microcystis* system with inoculation densities of 1.9×10^6 and 1.9×10^7 cfu/mL, resulting in the death of *M. aeruginosa* NIES-843. However, the population dynamics of strain B50 was a bell-shaped curve at low inoculation densities and no lytic effect could be observed. Results of physiological responses suggested that the lytic efficiency may be mediated through inhibition of metabolism and production of reactive oxygen species.

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Keywords *Bacillus* sp. · Gene expressions · Lytic characteristics · *Microcystis aeruginosa* · Oxidative stress · Photosynthesis inhibition

Introduction

Water blooms formed by cyanobacteria have posed a worldwide environmental threat in recent decades. In China, more than 60 % of the lakes are eutrophied and suffered from harmful algal blooms (HABs), in which *M. aeruginosa* is one of the dominant cyanobacterial species (Pan et al. 2006) and is also the most important species for the production of hepatotoxic microcystins (Song et al. 2007). The toxicity of microcystins is mediated through the inhibition of eukaryotic protein phosphatases 1A and 2A in liver cells, and causes hepatotoxicity leading to liver cancer and tumors in humans and wildlife (Carmichael 1995). Therefore, the control of HABs mediated by *M. aeruginosa* is crucial for maintaining safe water supplies worldwide including China. Several methods such as chemical algicides and physical interventions have been proposed and implemented to control harmful algal blooms, but most of them were either impracticable or non-effective because of high cost or subsequent secondary pollution issues (Anderson 1997). An alternative approach for the elimination and control of *M. aeruginosa* can be the application of biocidal agents. Bacteria are the main group of microorganisms showing lytic effect against *M. aeruginosa*, though some viruses and fungi have also been demonstrated to be able to kill *M. aeruginosa* (Tucker and Pollard 2005; Wang et al. 2010).

During the past decades, bacteria isolated with algicidal activity towards *M. aeruginosa* are mainly assigned to the genera *Alcaligenes*, *Pseudomonas*, *Bacillus*, *Streptomyces*

and *Rhodococcus* (Manage et al. 2000; Kodani et al. 2002; Mu et al. 2007; Kim et al. 2007; Lee et al. 2010). As a promising and environmentally friendly way to control water blooms, algicidal bacteria have received intensive studies. However, such studies mainly focused on the isolation and identification of algicidal bacteria and the lytic characters, and few studies attempted to elucidate the lytic mechanism of algicidal bacteria against *M. aeruginosa*. The inhibitory effects of algicidal bacteria against *M. aeruginosa* occurred through direct cell contact (Manage et al. 2000) and/or were mediated by extracellular substances (Mu et al. 2007). Identification of lytic substances is an important step to elucidate the lytic mechanism of algicidal bacteria against algae, but the lytic mechanism can hardly be revealed with the information of lytic substances alone. For example, harmane (1-methyl-β-carboline) was identified as a lytic substance responsible for the inhibitory effect of an algicidal bacterium *Pseudomonas* sp. K44-1 against cyanobacteria (Kodani et al. 2002), but it is still not known how this substance inhibits the growth of cyanobacteria. The physiological changes of the algae under the stress of algicidal bacteria are useful information for understanding the lytic mechanism, but till now, such information on physiological responses of the algae is very limited.

The lytic effects of algicidal bacteria against algae are density dependent of the algicidal bacteria and/or algae, indicating that lytic processes involve the interactions between algicidal bacteria and algae. However, the information about the interactions between algicidal bacteria and the algae in the lytic processes is currently not available.

In this study, an algicidal bacterial strain was isolated from the Lake Donghu of China and identified as *Bacillus* sp. (GenBank: HM543166). The interactions of this algicidal bacterium with *M. aeruginosa* were then examined for the lytic characteristics and the algal physiological responses. The whole research works were carried out from 2009 to 2011 in the laboratory of Biology of Harmful Algae, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, P. R. China.

Materials and methods

Algal strain and culture conditions

Microcystis aeruginosa NIES-843 was kindly provided by National Institute of Environmental Science, Japan. It was grown in CT liquid medium (pH 8.2) (Ichimura 1979), under a 12:12 light/dark (L/D) cycle with a light intensity of 30 μmol photons/(s m^2) provided by cool white fluorescent tubes at 28 ± 1 °C.

Isolation of algicidal bacterium

Water samples were collected from Bay Bitan, Lake Donghu, Hubei province, China, during the decline phase of *M. aeruginosa* bloom. Samples were serially diluted and spread on solid CT medium with modification (tryptone, yeast extract and sodium citrate were added to CT medium at a final concentrations of 0.2, 0.1 and 2 g/L, respectively), and then incubated for 3 days at 28 ± 1 °C. Bacterial colonies were inoculated to liquid modified CT medium, and the cultures of each isolate were added into exponential-phase *M. aeruginosa* NIES-843 cultures at a ratio of 10 % (v/v). In the control treatment, 10 % modified CT medium was added. All treatments were cultured at 28 °C and with 80 rpm shaking, under 30 μmol photons/(s m^2) on a 12:12 L/D cycle. Prior to the present experiments, it was established in this laboratory that 10 % modified CT medium had no obvious effect on the growth and Chl *a* content of *M. aeruginosa* NIES-843 during the 5 days of incubation afterward. The cultures that caused the death of *M. aeruginosa* NIES-843 were regarded as algicidal bacteria.

Identification of algicidal bacterium

The genomic DNA of the algicidal bacteria was extracted using the method described by Lin et al. (2010). The PCR amplification reactions of 16S rDNA were performed on an MyiQ™ PCR Detection System (BIO-RAD, USA) under the following conditions: one cycle at 94 °C for 3 min, followed by 30 cycles of 94 °C for 45 s, 56 °C for 45 s, 72 °C for 1.5 min using primers 8F (5-AGAGTTTGATC CTGGCTCAG-3) and 1492R (5-GGTTACCTTGTTA CGACTT-3) (Vickerman et al. 2007). PCR products were confirmed before being sequenced by BGI Co., Ltd (Wuhan, China).

Determination of chlorophyll *a* contents

Cells of *M. aeruginosa* NIES-843 were harvested by centrifugation at 10,000×*g* for 10 min, and then immersed in liquid nitrogen to break the cells. Chlorophyll *a* (Chl *a*) was extracted with 80 % acetone in the darkness at 4 °C. Absorption was measured at 663 and 645 nm using spectrophotometer, and Chl *a* contents were calculated according to the method of Richards and Thompson (1952).

Lytic fractions determination

Algicidal bacterium *Bacillus* sp. strain B50 was incubated in the modified CT medium at 28 °C for 24 h with shaking at 250 rpm, and then subject to the following treatments:

(1) centrifuged at 12,000×*g* for 10 min, and the obtained pellet was re-suspended with sterilized CT medium to reach a cell density of more than 10^9 cfu/mL; (2) supernatants from the above treatment procedure were filtered through 0.22-μm cellulose acetate membrane filter; (3) the cell suspensions of the above treatment were treated by sonication for cell breakage, and then filtered through 0.22-μm membrane filter; (4) the filtrate of the *Bacillus* sp. B50 cultures were autoclaved at 121 °C for 20 min. All these above described treatments were added separately into exponential-phase *M. aeruginosa* NIES-843 cultures at a ratio of 10 % (v/v). In the control treatment, 10 % modified CT was added. All treatments were cultured at 28 °C for 3 days with shaking at 80 rpm under 30 μmol photons/(s m^2) on a 12:12 L/D cycle. Each treatment was replicated three times. The algicidal efficiency was assessed according to reduction of Chl *a* and was compared with the control as described in Eq. (1) below.

$$\text{Algicidal efficiency}\,(\%) = (1 - \text{Chl}a_{\text{treatment}}/\text{Chl}a_{\text{control}}) \times 100 \quad (1)$$

Relationship between inoculating density of algicidal bacteria and algicidal efficiency

The exponential phase cultures of *Bacillus* sp. B50 were harvested by centrifugation at 12,000×*g* for 10 min, and dilutions of culture were made to 1.9×10^8, 1.9×10^7 and 1.9×10^6 cfu/mL, respectively, with autoclaved CT medium. Bacterial suspensions of active culture (10 ml) were inoculated into 250-mL conical flasks containing 90 mL of exponential-phase *M. aeruginosa* NIES-843, corresponding to actual inoculating densities of 1.9×10^7, 1.9×10^6 and 1.9×10^5 cfu/mL, respectively. All treatments were cultured at 28 °C with 80 rpm shaking under 30 μmol photons/(s m^2) on a 12:12 L/D cycle. Bacterial suspension was substituted with sterilized CT medium in the control treatments. The initial Chl *a* concentration was set at approximately 0.56 mg/L. The algicidal effects were determined according to reduction of Chl *a* compared with the control. Each treatment was replicated three times. The axenic cultures of *M. aeruginosa* NIES-843 can not form bacterial colony unit on LB agar plates, however, algicidal bacterium *Bacillus* sp. can form colony units on LB agar plates. The population dynamics of *Bacillus* sp. B50 cell numbers in all treatments could be determined using series dilution and spread plating method on LB agar plates. For determination of *Bacillus* sp. B50 spores, the mixtures of all treatments were incubated in a water bath at 80 °C for 15 min to kill the vegetative cells, and then aliquot from treatments was spread onto agar plates containing modified CT medium.

Algal physiological responses

The cultures of *Bacillus* sp. B50 were inoculated into the cultures of *M. aeruginosa* NIES-843 at a ratio of 10 % (v/v) following the procedures mentioned above. The initial inoculating density of *Bacillus* sp. B50 was 1.9×10^5 and 1.9×10^7 cfu/mL, and the initial Chl *a* concentration was 0.56 mg/L.

Determination of maximum electron transport rate (ETR_{max}) of Photosystem II (PS II)

ETR_{max} was measured using a pulse-amplitude-modulated fluorescence monitoring system (PAM, Walz, Effeltrich, Germany). The numerical values of chlorophyll fluorescence of samples exposed to 12 intensities of actinic light increasing from 0 to 1,265 μmol photons/(s m^2) photosynthetically active radiation were recorded during a 3-min time series.

Measurement of intracellular reactive oxygen species levels

The intracellular reactive oxygen species (ROS) levels in *M. aeruginosa* NIES-843 were detected using 2,7-dichlorofluorescein diacetate (Sigma Chemical, St. Louis, MO, USA) based on the method described by Hong et al. (2008). The fluorescence intensity of 2,7-dichlorofluorescein was obtained using a microplate reader (Molecular Device, M2, Union City, CA, USA). Excitation and emission wavelengths were 485 and 530 nm, respectively.

Determination of gene expression profiles

The expressions of six genes (Table 1) in *M. aeruginosa* NIES-843 under the stress of algicidal bacterium inoculation were determined by qPCR on samples from the 1st and 2nd days of incubation. Total RNA extraction and reverse transcription were performed according to the methods described by Shao et al. (2009). In brief, the cells of *M. aeruginosa* NIES-843 were harvested and resuspended in Trizol reagent (Invitrogen, Carlsbad, CA, USA), and homogenized with a mini-beadbeater. Total RNA was extracted following the Trizol reagent manual. Total RNA was digested with RQ1 RNase-free DNase (Promega, Madison, WI, USA). These DNase-treated RNA were reverse transcripted to cDNA using random primers $p(dN)_9$ and a reverse transcriptase kit (Generay, Shanghai, China). The amplification reactions were performed on an MyiQ™ qPCR Detection System (BIO-RAD, Hercules, CA, USA) under the following conditions: one cycle at 95 °C for

Table 1 Targeted genes used for transcription analysis in this study

Gene name	Gene function	Reference
prx	Encodes peroxiredoxin, a protein belongs to the thiol oxidation system	Latifi et al. (2007)
mcyB	Encodes McyB, a protein involves in the synthesis of microcystins	Pearson and Neilan (2008)
psbA	Encodes D1 protein of PS II	Aro et al. (1993)
grpE	Encodes the heat-shock protein GrpE, a protein belonging to DnaK-DnaJ-GrpE chaperone system	Gamer et al. (1992)
fabZ	Encodes β-hydroxyacyl acyl carrier protein dehydratase, a protein involves in the synthesis of fatty acids	Swarnamukhi et al. (2006)
ftsZ	Encodes FtsZ, a protein involved in cell division	Mazouni et al. (2004)

Table 2 PCR primers designed for qPCR analysis

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
16S *rrn*	GGACGGGTGAGTAACGCGTA[a]	CCCATTGCGGAAAATTCCCC[b]
Prx[c]	GCGAATTTAGCAGTATCAACACC	GCGGTGCTGATTTCTTTTTTC
psbA[c]	GGTCAAGARGAAGAAACCTACAAT	GTTG AAACCGTTGAGGTTGAA
mcyB[d]	CCTACCGAGCGCTTGGG	GAAAATCCCCTAAAGATTCCTGAGT
ftsZ	TCGCTGCTATTTCCTCGC	TGACTTCTCCCTGCATTTTCT
grpE[c]	CGCAAACGCACAGCCAAGGAA	GTGAATACCCATCTCGCCATC
fabZ[c]	TGTTAATTGTGGAATCCATGG	TTGCTTCCCCTTGCATTTT

[a] Primer modified based on the reference reported by Urbach et al. (1992)

[b] Primer sequence obtained from Nübel et al. (1997)

[c] Primer sequence obtained from Shao et al. (2009)

[d] Primer sequence obtained from Kurmayer and Kutzenberger (2003)

3 min, followed by 40 cycles of 95 °C for 15 s, 59 °C for 30 s, 72 °C for 15 s. PCR primers used in this study are listed in Table 2. Gene expression data from the qPCR were evaluated using Ct values. The 16S rRNA gene was used as a housekeeping gene to normalize the expression levels of target genes (Bustin 2000). The induction ratio was calculated using $2^{-\Delta\Delta Ct}$.

$$\Delta\Delta Ct = (Ct_{\text{target gene}} - Ct_{16S\ rrn})_{stress} - (Ct_{\text{target gene}} - Ct_{16S\ rrn})_{control} \quad (2)$$

Statistics

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

Results and discussion

Isolation and identification of the algicidal bacterium

Among the 87 isolates, strain B50 showed the highest lytic efficiency against *M. aeruginosa* NIES-843. The 16S rDNA sequence homology of strain B50 (GenBank: HM543166) showed high similarity with many strains of *Bacillus* in NCBI database, and it reached 100 % with *Bacillus pumilus.*

Lytic fractions of *Bacillus* sp. B50 cultures

The treatments of *Bacillus* sp. B50 cultures, cells suspensions, cell free cultures and autoclaved cell-free cultures all showed apparent lytic effect on *M. aeruginosa* NIES-843, but the treatment of cell debris did not show any lytic effect (Fig. 1). In general, it is known that there are two lytic models for algicidal effects from bacteria: direct cell contact (Manage et al. 2000) and/or mechanisms mediated by extracellular substances (Mu et al. 2007). The cell-free culture filtrate of *Bacillus* sp. B50 showed lytic effect against *M. aeruginosa* NIES-843, indicating that the lytic effect was mediated by extracellular substances produced by the lytic bacteria. Since the cell debris did not show any lytic effect against *M. aeruginosa* NIES-843, the lytic substances may mainly extracellular in nature, outside of cells rather than accumulated inside the cells. Our previous work also showed that *Bacillus* sp. B50 could grow in the *Microcystis* culture. Synthesis and excretion of lytic substances de novo are a

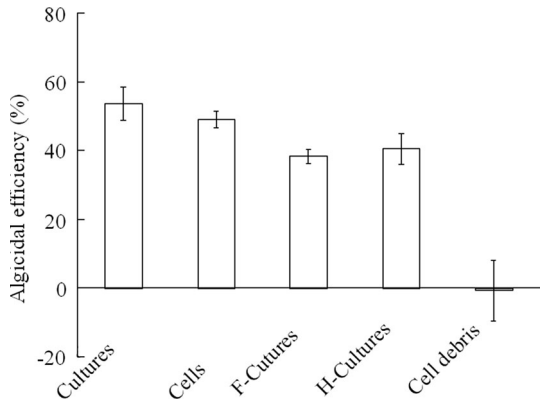

Fig. 1 Lytic efficiencies of different fractions of *Bacillus* sp. B50 cultures. Cultures: Cultures of *Bacillus* sp. B50; Cells: cell suspensions of *Bacillus* sp. B50; F-Cultures: *Bacillus* sp. B50 cultures filtered through 0.22-μm membrane filter; H-Cultures: autoclaved filtrates of *Bacillus* sp. B50 cultures; Cell debris: Cell debris of *Bacillus* sp. B50 culture obtained through centrifugation. Average values ± standard deviation ($n = 3$)

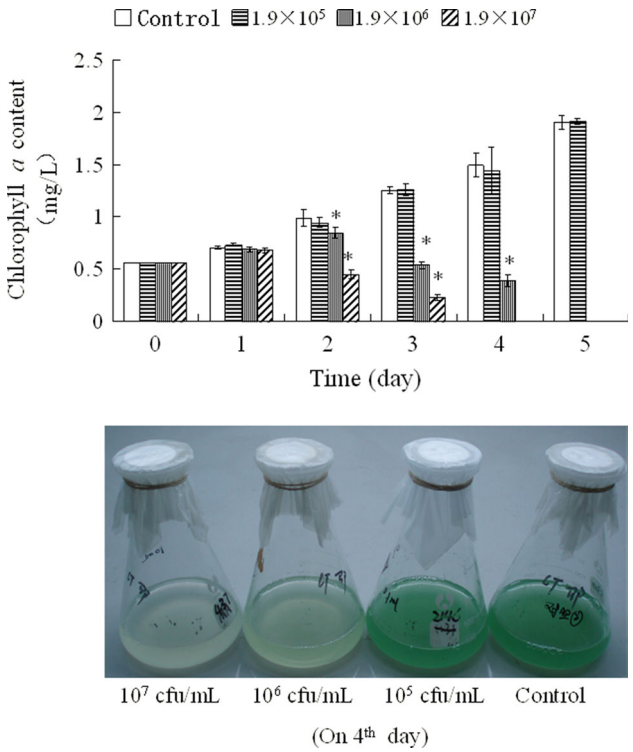

Fig. 2 Influence of algicidal bacterial inoculating density on algicidal efficiency. Average values ± standard deviation ($n = 3$). *$p < 0.05$

plausible reason for the results observed in which the re-suspended cells of *Bacillus* sp. B50 also showed lytic effect against *M. aeruginosa* NIES-843. The lytic efficiency of autoclaved cell-free culture filtrate showed that there was no significantly difference from the treatment of

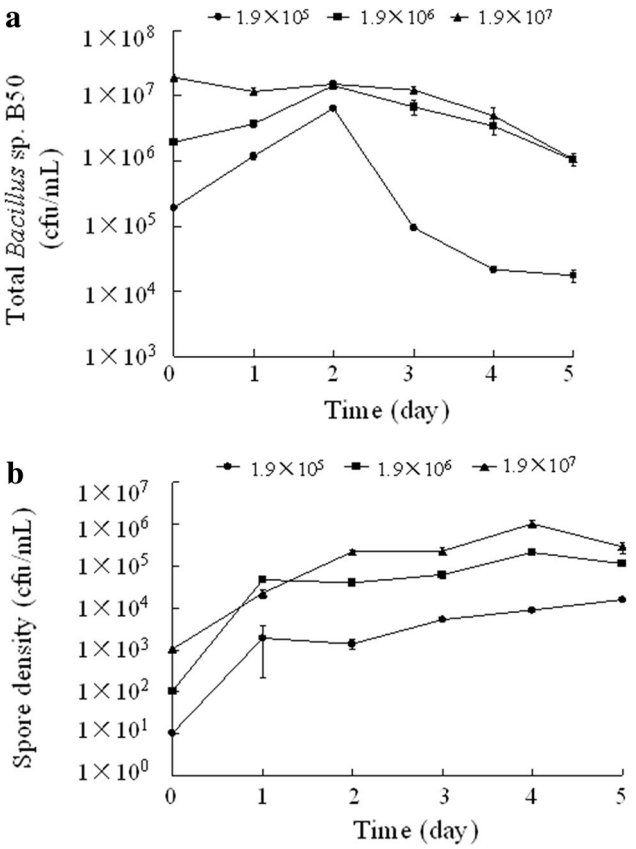

Fig. 3 Population dynamics of *Bacillus* sp. B50 cell numbers (colony forming unit) (**a**) and spores (**b**) in bacteria-algae systems. Average values ± standard deviation ($n = 3$)

non-autoclaved cell-free (active) culture filtrate, indicating that the lytic substances involved are heat-resistant ones.

Relationship between inoculating density of algicidal bacterium and algicidal efficiency

Inoculation series of algicidal bacterium *Bacillus* sp. B50 showed variable algicidal efficiency (Fig. 2). There was no lytic effect against *M. aeruginosa* NIES-843 at an inoculating level of 1.9×10^5 cfu/mL, but the growth of *M. aeruginosa* NIES-843 was significantly inhibited at the inoculating levels of 1.9×10^6 and 1.9×10^7 cfu/mL, and the cells of *M. aeruginosa* NIES-843 were completely killed by *Bacillus* sp. B50 on the 4th day of incubation at 1.9×10^7 cfu/mL and on the 5th day at 1.9×10^6 cfu/mL. Previous work indicated that lytic effects of algicidal bacteria were always cell density dependent. Kang et al. (2005) reported that the algicidal bacterium *Pseudomonas putida* HYK0203-SK02 showed lytic effect at 1×10^6, 1×10^7 and 1×10^8 cfu/mL, but no lytic effect was observed at $\leq 1 \times 10^5$. Similar phenomena were also observed in the algicidal bacterium *Myxococcus fulvus* (Fraleigh and Burnham 1988). These phenomena were also in agreement with our results.

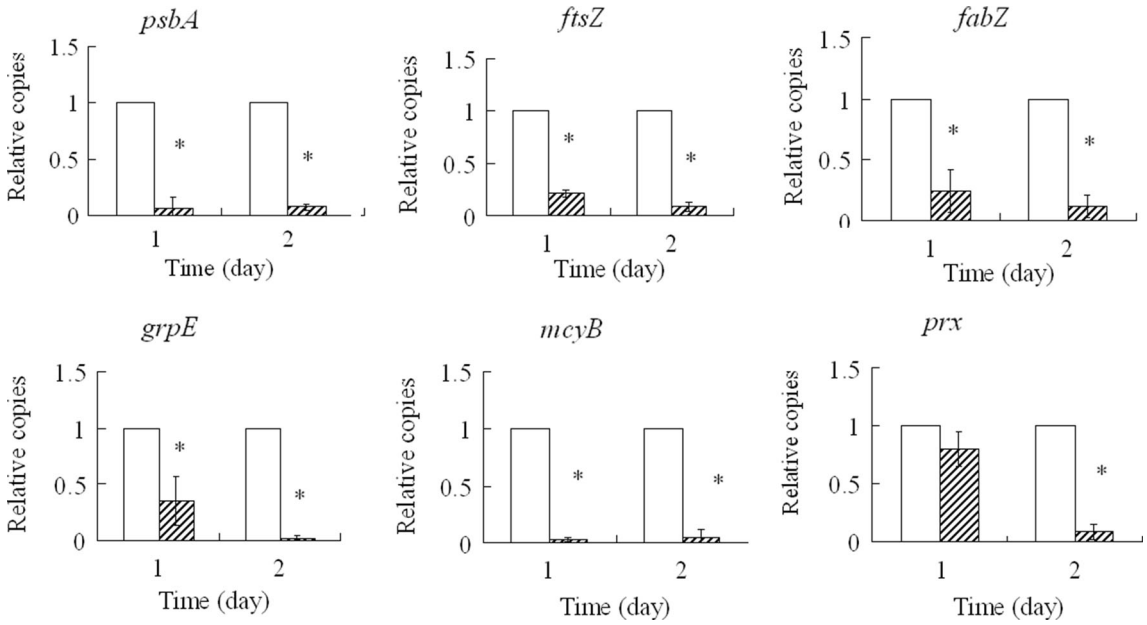

Fig. 4 Relative normalized expressions of six genes (*psbA*, *ftsZ*, *fabZ*, *grpE*, *mcyB* and *prx*) of *M. aeruginosa* NIES-843 under the stress of algicidal bacterium *Bacillus* sp. B50 (1.9×10^7 cfu/mL) after normalization. *No fill*: control, *shaded*: *Bacillus* sp. B50 inoculation. Average values ± standard deviation ($n = 3$). *$p < 0.05$

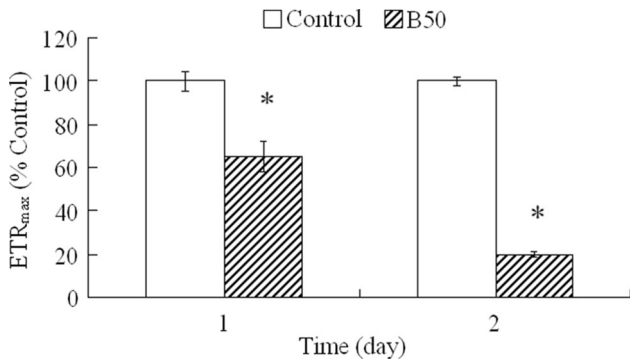

Fig. 5 Effects of inoculation (1.9×10^7 cfu/mL) of algicidal bacterium *Bacillus* sp. B50 on maximum electron transport rate (ETR_{max}) of *M. aeruginosa* NIES 843 PS II. Average values ± standard deviation ($n = 3$). B50: *Bacillus* sp. B50 inoculation. *$p < 0.05$

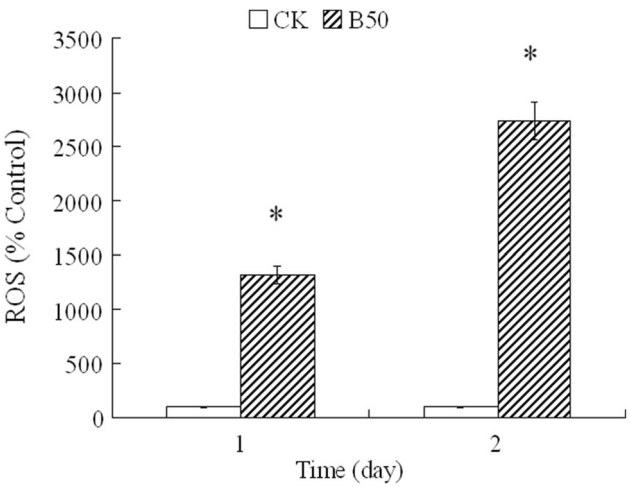

Fig. 6 Effects of inoculation (1.9×10^7 cfu/mL) of algicidal bacterium *Bacillus*. sp. B50 on intracellular ROS levels of *M. aeruginosa* NIES-843. B50: *Bacillus* sp. B50 inoculation; CK: Control check. Average values ± standard deviation ($n = 3$). *$p < 0.05$

Population dynamics of *Bacillus* sp. B50 cells and its spores

Population dynamics of *Bacillus* sp. B50 cells and its spores are shown in Fig. 3. The cell density of *Bacillus* sp. B50 increased to 6.3×10^6 cfu/mL on the 2nd day of incubation at an inoculating level of 1.9×10^5 cfu/mL, but it decreased rapidly to the level of 1.7×10^4 cfu/mL on the 5th day. The cell density of *Bacillus* sp. B50 maintained a relative high level ($>3 \times 10^6$ cfu/mL) during the first 4 days of incubation at an inoculating level of 1.9×10^6 and 1.9×10^7 cfu/mL, and then they decreased to about 1×10^6 cfu/mL on the 5th day. The spore densities of *Bacillus* sp. B50 in all these three inoculating levels continued to increase for the first 4 days, reaching 8.8×10^3, 2.2×10^6 and 1.0×10^6 cfu/mL, respectively.

It is possible that low population density of *Bacillus* sp. B50 cells could not synthesize and excrete enough lytic substances into the cultures to cause the death of *M. aeruginosa* NIES-843 because no lytic effect was detected at 1.9×10^5 cfu/mL. Our previous work indicated that *Bacillus* sp. B50 could not grow in the CT medium (unpublished data), but results of the current study indicated that *Bacillus* sp. B50 could grow in the cultures of *M. aeruginosa* NIES-843 using CT medium since the cell

density of *Bacillus* sp. B50 increased rapidly in the first 2 days of incubation at an inoculation level of 1.9×10^5 cfu/mL (Fig. 3a). This result indicated that dissolved organic materials from *M. aeruginosa* NIES-843 were essential for the growth of *Bacillus* sp. B50 in CT medium. Similar phenomena were also observed for the algicidal bacterial *Cytophaga* strain 41-DBG2 (Mayali and Doucette 2002). It is interesting to note the rapid decrease of the cell density of *Bacillus* sp. B50 from the 2nd day to the 5th day after inoculation of 1.9×10^5 cfu/mL (Fig. 3a). Obviously, it was caused by neither the accumulation of metabolic waste products of *Bacillus* sp. B50 nor the depletion of nutrients because the cell density maintained at high levels in the following 4 days after inoculation at the levels of 1.9×10^6 and 1.9×10^7 cfu/mL. One possible explanation is that *Bacillus* sp. B50 induced *M. aeruginosa* NIES-843 to synthesize some anti-bacteria substances, and then these substances inhibited the growth of *Bacillus* sp. B50. Such implication involving inter-organismal interaction rather than one direction action (algicidal bacteria affect algae) exists in the bacteria–algae systems.

Cyanobacteria can synthesize many kinds of cyanotoxins (Welker and von Döhren 2006), but only a few, e.g. microcystins are well studied. In the bacteria–algae system, microcystins are unlikely to be the anti-bacterial substances based on the following two reasons: (1) previous work showed that microcystins inhibit eukaryotic protein phosphatases 1A and 2A (Carmichael 1995) but showed no inhibitory effect on prokaryotic phosphatases (Kennelly and Potts 1999), though adverse effect on bacteria was also observed under the conditions of unrealistic high concentrations (Yang et al. 2009) and (2) microcystins are mainly accumulated within the cells (Juttner and Luthi 2008) and then released to the environment after cell lysis. *Bacillus* sp. B50 is not a microcystins-degrading bacterium (unpublished data), but the cell density of this algicidal bacterium could be maintained at high levels during the lytic process with inoculation of 1.9×10^6 and 1.9×10^7 cfu/mL. It is speculated that other active compounds rather than microcystins may actually cause the decrease in cell density of *Bacillus* sp. B50. The cell density of *Bacillus* sp. B50 showed an increase during the first 2 days of incubation at inoculation of 1.9×10^5 cfu/mL, but then a rapid decrease from the 2nd day to the 5th day (Fig. 3a). These results collectively indicate that the synthesis of this inhibitory substance is inducible, and *Bacillus* sp. B50 at low inoculating level can induce/increase synthesis of this substance, and then accumulated to level to inhibit the growth of *Bacillus* sp. B50. However, at high inoculating level, cells of *M. aeruginosa* NIES-843 are being killed by this algicidal bacterium before adequate anti-bacteria substances have been synthesized and accumulated in the culture.

Algal physiological responses

ETR_{max}, ROS and the expressions of six genes (*psbA*, *ftsZ*, *fabZ*, *grpE*, *mcyB* and *prx*) of *M. aeruginosa* NIES-843 were examined under the stress of algicidal bacterium inoculated at 1.9×10^5 and 1.9×10^7 cfu/mL. Compared with the controls, there was no significant difference on ETR_{max}, ROS and the expressions of the six targeted genes at the inoculating level of 1.9×10^5 (data not shown). In contrast, when the inoculating level was 1.9×10^7, the expression of the six genes and the ETR_{max} were depressed significantly, but the ROS in the cells of *M. aeruginosa* NIES-843 boosted drastically (Figs. 4, 5, 6).

The six targeted genes in this study are involved in synthesis of microcystins, fatty acid, photosynthesis system, cell division, an antioxidant system and repairing of protein aggregation (Table 1). The expressions of all six genes were significantly inhibited by B50 extracellular materials suggesting that the metabolisms of *M. aeruginosa* NIES-843 were significantly inhibited by this algicidal bacterium. The ETR_{max} reflects the electron transport rate of PS II, and the decreased electron transport rate indicated that more excitation energy was transferred to ROS such as singlet oxygen. The algicidal bacterium *Bacillus* sp. B50 could decrease ETR_{max} of *M. aeruginosa* NIES-843. This may be the reason for the boost of ROS in the cells of *M. aeruginosa* NIES-843 under the stress of *Bacillus* sp. B50 inoculation. High levels of ROS can react with DNA, unsaturated fatty acids and other bio-molecules (Griffiths 2005), resulting in the death of *M. aeruginosa* NIES-843 induced by *Bacillus* sp. B50.

Conclusion

An algicidal bacterium identified as *Bacillus* sp. shows lytic effect against *M. aeruginosa* NIES-843, possibly mediated by its heat-stable extracellular substances. The cell density of *Bacillus* sp. B50 could be maintained at high level in the bacteria-algae system and caused the death of *M. aeruginosa* NIES-843 with inoculation densities of 1.9×10^6 and 1.9×10^7 cfu/mL. Physiological responses of *M. aeruginosa* NIES-843 suggested that metabolism inhibition and ROS elevation may be the lytic mechanism of *Bacillus* sp. B50 involved against cyanobacteria in this study.

Acknowledgments The research was supported by National Natural Science Foundation of China (No. 21107024), Hunan Provincial Natural Science Foundation of China (10JJ6045), and the Foundation of Furong Scholar project of Hunan Province. Professor Joe Lepo (University of West Florida) provided language assistance and useful suggestions in an early draft of this manuscript.

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