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ARTICLE

Exploration of the antioxidant system and photosynthetic system of a marine algicidal *Bacillus* and its effect on four harmful algal bloom species

Shaoling Hou, Wanjiao Shu, Shuo Tan, Ling Zhao, and Pinghe Yin

Abstract: A novel marine bacterium, strain B1, initially showed 96.4% algicidal activity against *Phaeocystis globosa*. Under this situation, 3 other harmful algal species (*Skeletonema costatum*, *Heterosigma akashiwo*, and *Prorocentrum donghaiense*) were chosen to study the algicidal effects of strain B1, and the algicidal activities were 91.4%, 90.7%, and 90.6%, respectively. To explore the algicidal mechanism of strain B1 on these 4 harmful algal species, the characteristics of the antioxidant system and photosynthetic system were studied. Sensitivity to strain B1 supernatant, enzyme activity, and gene expression varied with algal species, while the algicidal patterns were similar. Strain B1 supernatant increased malondialdehyde contents; decreased chlorophyll *a* contents; changed total antioxidant and superoxide dismutase activity; and restrained *psbA*, *psbD*, and *rbcL* genes expression, which eventually resulted in the algal cells death. The algicidal procedure was observed using field emission scanning electron microscopy, which indicated that algal cells were lysed and cellular substances were released. These findings suggested that the antioxidant and photosynthetic system of these 4 algal species was destroyed under strain B1 supernatant stress. This is the first report to explore and compare the mechanism of a marine *Bacillus* against harmful algal bloom species of covered 4 phyla.

Key words: harmful algal blooms, algicidal *Bacillus*, gene expression, morphologic damage, oxidative damage.

Résumé : Une nouvelle bactérie marine, la souche B1, a tout d'abord démontré une activité algicide de 96,4 % envers *Phaeocystis globosa*. En vertu de ce constat, on a choisi 3 autres espèces d'algues nuisibles (*Skeletonema costatum*, *Heterosigma akashiwo* et *Prorocentrum donghaiense*) afin d'étudier l'effet algicide de la souche B1, et on a relevé des activités algicides de 91,4 %, 90,7 % et 90,6 %, respectivement. Afin d'examiner le mécanisme algicide de la souche B1 agissant contre ces 4 espèces d'algues nuisibles, on a caractérisé le système antioxydant et photosynthétique. La sensibilité au surnageant de la souche B1, l'activité enzymatique et l'expression génique ont varié selon l'espèce d'algue, tandis que les profils algicides étaient comparables. Le surnageant de la souche B1 a haussé la teneur en malondiadéhyde, abaissé la teneur en chlorophylle *a*, fait varier l'activité antioxydante totale et celle liée à la superoxyde dismutase, et restreint l'expression génique de *psbA*, *psbD* et *rbcL*, ce qui a conduit à la mort des cellules algiques. Le processus algicide a été observé par microscopie électronique à balayage à émission de champ, lequel a révélé que les cellules d'algues avaient été lysées et que des substances cellulaires se sont déversées. Ces trouvailles indiquent que le système antioxydant et photosynthétique de ces 4 espèces d'algues a été détruit sous l'effet du stress imposé par le surnageant de la souche B1. Il s'agit du premier compte-rendu examinant et comparant la mécanique algicide d'un genre de *Bacillus* marin qui s'attaque à des espèces d'algues nuisibles prolifératives appartenant à 4 phylums. [Traduit par la Rédaction]

Mots-clés : proliférations d'algues nuisibles, *Bacillus* algicide, expression génique, dommage morphologique, dommage oxydatif.

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Introduction

In recent years, there have been more frequent outbreaks of harmful algal blooms (HABs). These HABs have aggravated pollution, caused economic losses, and been associated with diseases hazardous to human health (Hallegraeff 1993; Briand et al. 2008; Mohamed et al. 2014). In particular, *Skeletonema costatum*, *Heterosigma akashiwo*, *Phaeocystis globosa*, and *Prorocentrum donghaiense* are notorious HABs species, causing frequent outbreaks in the Chinese coastal area (Mayali and Azam 2004; Zheng et al. 2013). Approaches involving the application of physical and chemical methods have been carried out to prevent or remove HAB occurrences (Wang et al. 2005; Costas and Lopez-Rodas 2006). However, these approaches are limited owing to nontarget toxicity and secondary pollution (Anderson 1997, 2009; Pierce et al. 2004). Biological methods including bacteria, fungi, virus, macrophytes, and protozoa (Nakashima et al. 2006; Su et al. 2007; Brussaard et al. 2007; Jeong et al. 2008; Graham and Strom 2010) have gained increasing attention. Algicidal bacteria are the main group of producers of extracellular active substances, and they have been widely studied for their ability to inhibit the growth of harmful algae (Furusawa et al. 2003; Amaro et al. 2005; Kim et al. 2008, 2009; Yang et al. 2013). Most of these reported bacteria have a relatively species-specific algicidal effect on algae. For example, Li et al. (2015) isolated *Bacillus* sp. strain Lzh-5 and demonstrated that its compounds showed strong algicidal activity against *Microcystis aeruginosa*. In a previous study, only a few algicidal bacteria showed a relatively wide host range and exerted activities on a variety of freshwater algae species (Liao and Liu (2014), but there are few reports indicating these bacteria have parallel algicidal effects against marine algal species of the 4 phyla covered.

Current studies about the algicidal mechanism have researched the change of malondialdehyde (MDA), antioxidant enzymes, and photosynthetic genes in algal cells (Shi et al. 2009; Zhang et al. 2013*a*). MDA is an important product of lipid peroxidation inside the cells, and thus reflects the degree of oxidative damage in algal cells. Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), can scavenge free radicals and catalyze antioxidant defense reactions so as to inhibit oxidative damage caused by the radicals (Elbaz et al. 2010). Photosynthesis relies on the transcript of photosynthetic genes in photosystems. The genes *psbA* and *psbD* encode 2 core proteins, D1 and D2, respectively, of photosystems (Marder et al. 1987), and *rbcL* encodes the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) (Asada et al. 1998). Their expressions are used to explore whether there was damage in the photosynthetic processes. Previous investigations mainly focused on the algicidal bacteria associated with the growth of *M. aeruginosa* and *Alexandrium tamarense* (Wang et al. 2012; Zhang et al. 2013*b*; Li et al. 2014). However, few reports have demonstrated the changes to and the characteristic of the antioxidant system and photosynthetic system for *S. costatum*, *H. akashiwo*, *P. globosa*, and *P. donghaiense* species, especially in terms of their expression of genes.

A novel marine bacterium, the algicidal *Bacillus* sp. strain B1, with indirect algicidal activity against *P. globosa*, was isolated from decayed algal blooms and identified by 16S rDNA sequence (Li 2012). We carried out studies to better understand and to compare the inhibitory mechanism of strain B1 supernatant on *S. costatum*, *H. akashiwo*, *P. globosa*, and *P. donghaiense*. Algicidal activity and basic characteristics of the antioxidant system and photosynthetic system for these 4 algal species were implemented during the algal lysis. The cell morphology was observed using field emission scanning electron microscopy (FESEM). In addition, the gene expressions of these algal species after exposure to strain B1 supernatant were also investigated. This is the first report to explore the algicidal effect of a marine algicidal bacterium on 4 harmful algae species at the level of gene expression.

Materials and methods

Four algal cultures and strain B1 supernatant

Algal cultures

Four algal species (*Skeletonema costatum*, *Heterosigma akashiwo*, *Phaeocystis globosa*, and *Prorocentrum donghaiense*) cultures were obtained from the Algal Culture Collection, Institute of Hydrobiology, Jinan University (Guangzhou, China). Cultures were prepared in f/2 medium under the light conditions of 12 h light : 12 h dark at 20 ± 1 °C (light incubator GXZ-0328). The test started when the concentration of algae reached approximately 10^7 cells/mL.

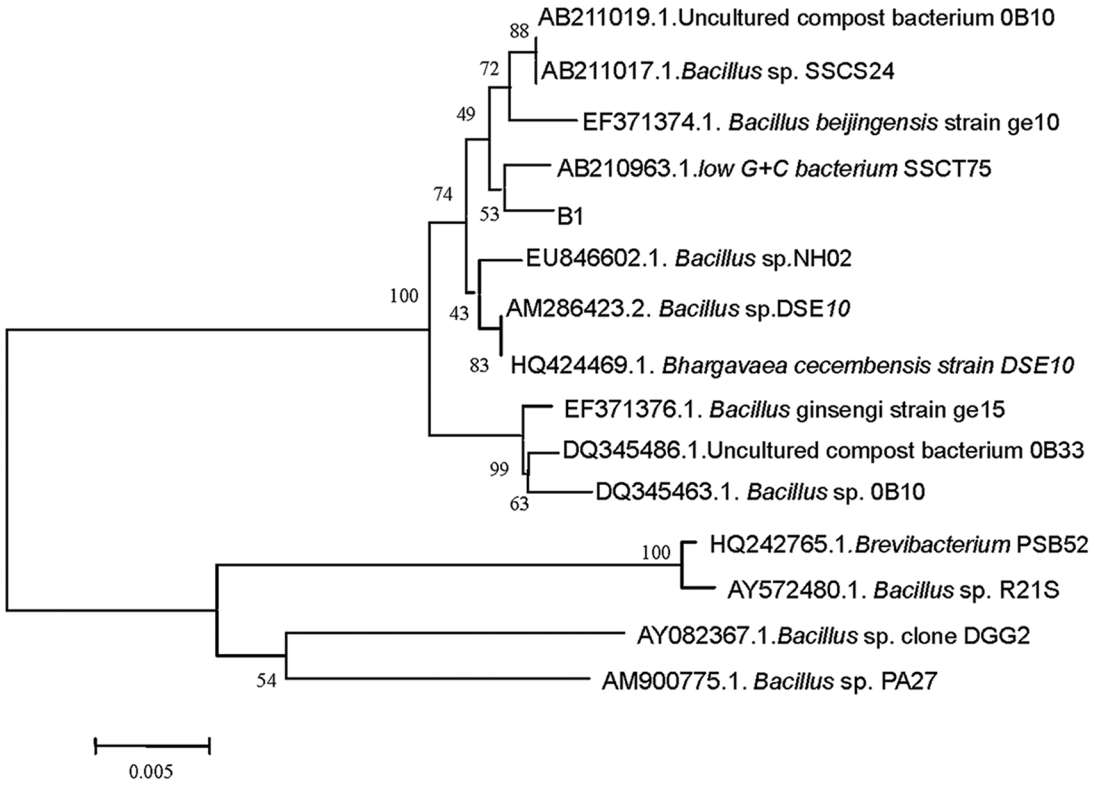
Bacterial strain and supernatant preparation

Strain B1 was previously isolated by our group and its GenBank accession number is JN 228893 (Fig. 1). Strain B1 was cultivated in 2216E medium at 30 °C (200 r/min) for 5 days, then centrifuged at 6000*g* for 10 min. The supernatant was collected after passing through a 0.22 μm pore size Millipore membrane and stored at 4 °C until use.

Determination of algicidal activity

Chlorophyll *a* was used to determine algicidal activity. When the algae grew to the logarithmic growth phase, strain B1 cell-free supernatant and 2216E liquid medium were added to the treatment groups at a proportion of 1.0% (*v*/*v*), and the culture conditions were the same as algal culture (see section Algal cultures above). Cultures without adding supernatant or bacterial media were prepared as controls. An aliquot of 20 mL of algal culture was removed daily and centrifuged at 4000*g* for 10 min to collect algal cells. The algal cells were stored in the dark at 4 °C for 24 h in 5 mL of acetone (95%). The acetone extract was centrifuged at 4000*g* for 10 min, and then the absorbance was measured at wavelengths of 665, 645, and 630 nm by a

Fig. 1. Phylogenetic tree based on 16S rDNA gene of strain B1 (Zhao et al. 2014). The tree was inferred using the neighbor-joining method. Bootstrap values were evaluated from 1000 replications.



721 Vis spectrophotometer. The concentration of chlorophyll *a* was calculated using the following formula:

$$\text{Chlorophyll a (mg/L)} = 11.6 \times A_{665} - 1.31 \times A_{645} - 0.14 \times A_{630}$$

and algicidal activity was calculated with the following formula (Hoshaw and Rosowski 1973):

$$\text{Algicidal activity (\%)} = (1 - N_t/N_c) \times 100\%$$

where N_t represents the chlorophyll *a* content of the treatment groups with supernatant, and N_c represents the chlorophyll *a* content of the control groups without added supernatant or bacterial media.

Oxidative stress analysis

Lipid peroxidation was measured by MDA, and antioxidant ability was assayed by enzyme (SOD, CAT, and POD) activities. When the algae grew to the logarithmic growth phase, strain B1 supernatant was added to the treatment groups at a proportion of 1.0% (*v*/*v*) but not to the control groups, and the culture conditions were the same as that of algal culture (see section Algal cultures above). The sample treatment method of algae for analysis was as follows: algal cells were collected by centrifugation at 6000*g* for 15 min at 4 °C; PBS (0.05 mol/L, pH 7.8) was added to the cells, which were then ground into a homogenate in an ice bath; then the supernatant of homogenate was collected at 4 °C and stored in the –70 °C until use.

MDA content was determined by means of the thiobarbituric acid (TBA) method using the MDA extinction coefficient of 155 L/(mmol·cm) to obtain its absorbance at a wavelength of 532 nm and its nonspecific absorbance at a wavelength of 600 nm. MDA content was expressed as nanomoles of MDA per milligram of protein (Zhang et al. 2014). The degree of lipid peroxidation was determined through the reaction of TBA with MDA (Shiu and Lee 2005). The total volume of the reaction mixture for the MDA assay was 4 mL, including 2.0 mL of TBA (0.6%) and 2.0 mL of the supernatant of the homogenate.

SOD activity was measured by using the nitro blue tetrazolium photoreduction method (Beauchamp and Fridovich 1971; Cakmak and Marschner 1992). The reaction mixture (6 mL) contained PBS (3 mL, 0.05 mol/L, pH 7.8), methionine (0.6 mL, 130 mmol/L), EDTA–Na_2 solution (0.6 mL, 100 μmol/L), riboflavin (0.6 mL, 20 μmol/L), nitro blue tetrazolium (0.6 mL, 750 μmol/L), H_2O_2 (0.5 mL, 30%), enzyme extract (0.1 mL), and the absorbance was measured at wavelengths of 560 nm. CAT was assayed by ultraviolet spectrophotometry within a mixed system (3 mL), containing H_2O_2 solution (0.3 mL, 0.1 mol/L), PBS (1.5 mL, 0.05 mol/L, pH 7.8), distilled water (1 mL), and enzyme extract (0.2 mL), and the absorbance was measured at a wavelength of 240 nm (Kumar et al. 2008; Zhao et al. 2012). POD was assayed according to the Guaiacol method

Table 1. Sequences of primer pairs used in real-time PCR.

Algae	Primer name	Forward sequence (5′–3′)	Reverse sequence (5′–3′)	Acc. No.
Phaeocystis globosa	*rbcL*	5′-GCTTGCTAACCTAACTGCTTCA-3′	5′-GGACGACCAAACTTATCCATTC-3′	NC021637
	psbA	5′-GCTTTTATCGCTGCACCTCC-3′	5′-CACCGTTGTATAACCACTCATCG-3′	NC021637
	psbD	5′-CTCCAGCAAATAGTATGGGTCA-3′	5′-GCAACAAACGTCCAAAGTCC-3′	NC021637
	18S rRNA	5′-CATTTGCCAGGGATGTTTTC-3′	5′-AAGTTTCAGCCTTGCGACC-3′	EU077556
Skeletonema costatum	*rbcL*	5′-AACGTGCTGAGTATGCTAAAG-3′	5′-CGGAAGTTAATACCATGATT-3′	AF015569
	psbA	5′-TCCCAAGTTCAAACGCTATC-3′	5′-ATTCCCACTCACGACCCA-3′	AY119761
	psbD	5′-GCATTTATCGCTTTACACGG-3′	5′-AAGAACCAACTTGCTTGACCT-3′	AY876246
	18S rRNA	5′-CATTTACCAAGGATGTTTT-3′	5′-AAGTTTCAGCCTTGCGACC-3′	JN676163
Heterosigma akashiwo	*rbcL*	5′-AGCGTGCTGACTATGCTAAAG-3′	5′-CGGAAGTTGATACCGTGATT-3′	NC010772
	psbA	5′-CAGCCAAGGTCGTGTAATCA-3′	5′-GCTAAGTCTAATGGGAAGTTGTG-3′	NC010772
	psbD	5′-ACTTTCGTCGCTTTACACGG-3′	5′-AAGAACCAACTTGCTTGACCT-3′	NC010772
	18S rRNA	5′-CATTTACCAAGGATGTTTTC-3′	5′-AAGTTTCAGCCTTGCGACC-3′	KP780271

with the reaction mixture (4 mL) of H_2O_2 solution (1.5 mL, 0.2%), guaiacol solution (1.0 mL, 0.2%), PBS (1.0 mL, 0.05 mol/L, pH 7.0), and enzyme extract (0.5 mL), and the decrease in absorbance was measured at a wavelength of 470 nm (Civello et al. 1995).

Electron microscopy analysis

Field emission scanning electron microscopy (FESEM) was used to observe the cellular morphology and compare the differences among the 4 kinds of algae after adding strain B1 supernatant (1.0%, *v*/*v*). Algal cells collected from algal cultures were first fixed with 2.5% glutaraldehyde overnight and then washed with PBS (0.1 mol/L, pH 7.2), dehydrated with gradient ethanol, passed through 100% acetone, and replaced with isoamyl acetate. Once fully dried, the samples were mounted on copper stubs and sputter-coated with gold palladium. The samples were observed with FESEM (ZEISS ULTRA™ 55, Germany).

RNA extraction and quantitative real-time PCR analysis

To find whether the strain B1 supernatant influence the thylakoid membrane electron transfer and the photosynthetic system, the algal photosynthetic gene expressions were determined by the quantitative real-time PCR (qRT-PCR). The algal culture conditions were the same as algal culture (see section Algal cultures above). Algal cultures were centrifuged at 2500*g* for 5 min to collect precipitate, and the total RNA was extracted following the Trizol Reagent (Life Technologies, 15596-018). Electrophoresis was used to check RNA integrity, and absorbance was measured at 260 and 280 nm to determine the RNA concentration and purity. Samples without reverse transcription were designed as negative controls to check for the possibility of amplification from contaminated genomic DNA. The reverse transcription step was taken in strict accordance with the TaKaRa PrimeScript™ RT Reagent kit (Perfect Real Time) instructions. The reaction conditions were run 15 min at 37 °C and 5 s at 85 °C. Primer pairs are listed in Table 1. The qRT-PCR using a Bio-Rad CFX96 Real-Time PCR System and SYBR premix EX Taq™ (2×) were run at the following conditions: 95 °C for 30 s, 45 cycles with 15 s at 95 °C, 20 s at 55 °C, and 20 s at 72 °C. Final concentrations were in a total volume of 10 μL, including 5 μL of SYBR premix EX Taq™ (2×), 0.5 μL of each primer (10 μmol/L), 0.5 μL of cDNA template, and 3.5 μL of dH_2O. Analysis of the melting curves verified the accuracy of each amplicon, and ABI prism SDS 7300 software was used to analyze the density of SYBR Green I and to determine the threshold cycle (Ct). The mRNA levels of the target genes in the microalgae incubated without B1 supernatant addition were used as calibrators. The reference gene of 18S rRNA was used to standardize the results, and the relative gene expression was quantified using the $2^{-\Delta\Delta C_T}$.

Statistical analysis

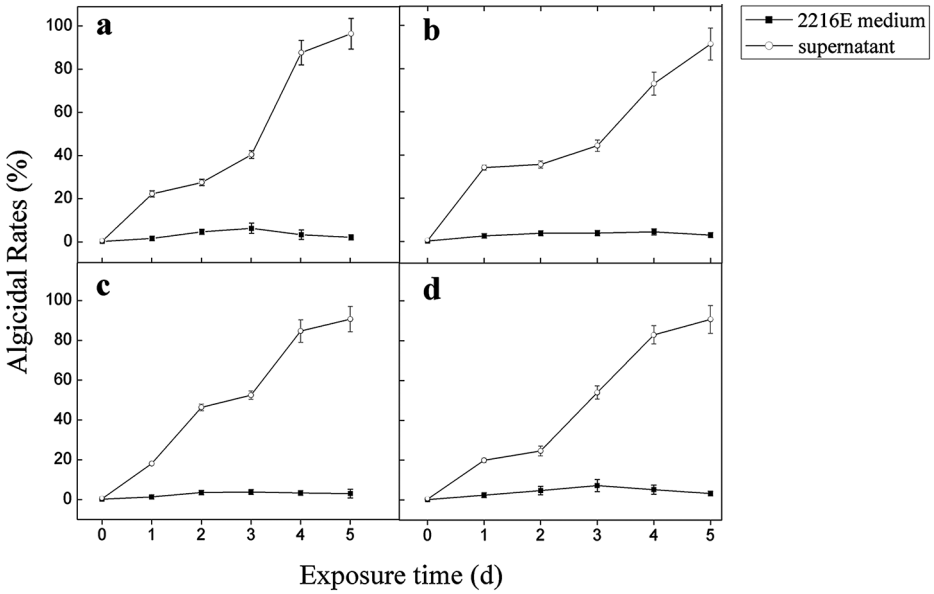
Data are presented as means ± standard error and evaluated by the repeated-measures ANOVA, with p values of <0.01 and <0.05. All statistics were performed with Origin 8.0 for Windows. All treatment groups were in triplicate.

Results

Algicidal effect

The algicidal activities of strain B1 supernatant inoculated into 4 algal species (*S. costatum*, *H. akashiwo*, *P. globosa*, and *P. donghaiense*) are illustrated in Fig. 2. Strain B1 exhibited an inhibitory effect on these 4 algal species, and the maximum algal-lytic activity could be summarized in the following order of sensitivity: *P. globosa* (96.4%) > *S. costatum* (91.4%) > *H. akashiwo* (90.7%) > *P. donghaiense* (90.6%). However, the addition of 2216E medium showed no effect on the growth of the 4 algal species compared with the controls. *Skeletonema costatum* (Fig. 2*b*) possessed 34.2% higher algicidal levels after adding strain B1 supernatant for 1 day. After 3 and 4 days of the experiment, the algicidal activity was significantly increased in *P. globosa* cultures (Fig. 2*a*) in comparison with cultures of other species (Figs. 2*c* and 2*d*), with the values increasing from 40.4% on Day 3 to 87.7% on Day 4. Moreover, high algicidal activity (>90%) was detected

Fig. 2. The algicidal activity on the 4 algal species (*a*) *Phaeocystis globosa*, (*b*) *Skeletonema costatum*, (*c*) *Heterosigma akashiwo*, and (*d*) *Prorocentrum donghaiense* exposed to 1.0% strain B1 supernatant and 2216E medium. All error bars correspond to the standard deviation.



when the treatment time was more than 5 days, as can be seen in Fig. 2.

Effect of cellular MDA contents and antioxidative enzyme activity

MDA can reflect the degree of lipid peroxidation, and it is used for evaluating the degree of cell damage. Figure 3 shows the MDA contents of strain B1 supernatant against the 4 algal species. During the first 3 days, the MDA contents increased significantly in all treatment groups. After 4 days, the MDA contents of *P. globosa* and *H. akashiwo* species reached their highest levels, which were 3.61 and 3.37 times higher ($p < 0.01$) than the controls, and then decreased after 5 days. However, the MDA contents of *S. costatum* ($p < 0.05$) and *P. donghaiense* treatment groups were the highest after 5 days, with levels at 3.62 times ($p < 0.05$) and 2.92 times that of the control values.

Activities of cellular enzymes SOD, CAT, and POD were determined to explore the cellular defense response of the 4 algal species induced by strain B1 supernatant (Fig. 4). After 2 days of treatment, SOD activity in *P. donghaiense*, *S. costatum*, *P. globosa*, and *H. akashiwo* reached maximum levels, rising by 1.46, 1.27 ($p < 0.05$), 1.17, and 1.18 times, respectively, over that of the controls (Fig. 4*a*). However, SOD activity significantly declined to lower levels for all treatment groups as the treatment time was prolonged. It is worth noting that *P. donghaiense* and *P. globosa* species responded more severely under the supernatant; their SOD activities decreased significantly and the lowest levels were 27.6% and 24.8% of the control levels after algal cells were treated for 5 days, while those of the *H. akashiwo* and *S. costatum* were 67.8% and 70.1% of the control levels, respectively.

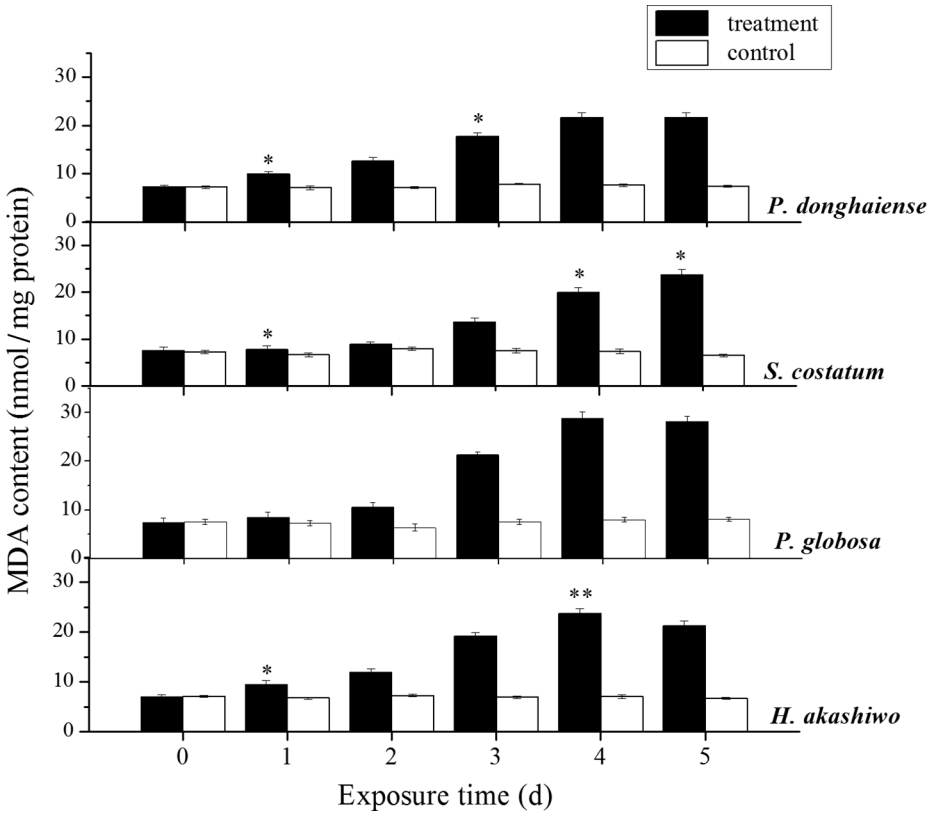
Figure 4*b* illustrates that the CAT activities of the 4 algal species showed a similar pattern, with the activities significantly increasing within 3 days. The activity values after treatment for 3 days reached their highest levels: 14.6, 9.55, 12.4, and 7.81 times higher ($p < 0.01$) in algal cells of *P. donghaiense*, *S. costatum*, *P. globosa*, and *H. akashiwo* treatment groups than in the controls. Subsequently, CAT activity decreased between 4 and 5 days exposure and eventually recovered to the initial level.

After 1 day of treatment, POD activities in algal cells of *P. donghaiense*, *S. costatum*, *P. globosa*, and *H. akashiwo* rose by 1.54, 2.53, 1.54, and 1.77 times over those of the controls (Fig. 4*c*). Then the POD activities for all treatment groups declined and were lower than those of the algal cells in the controls. Remarkably, the activities of *S. costatum* and *P. globosa* species reached their lowest levels in 3 days and exhibited a decrease of approximately 3.79% ($p < 0.01$) and 65.1%, respectively, compared with the controls. The lowest POD activities of the *P. donghaiense* and *H. akashiwo* treatment groups were acquired in 4 days of incubation and manifested a 7.47% and 8.23% ($p < 0.01$) decrease compared with the control. After more than 4 days, POD activities showed that longer exposure times did not cause a significant increase in the activity of this enzyme between the 4 kinds of algae.

Effects of cellular microstructure

FESEM analysis revealed morphological alterations of the cell surface in 4 kinds of algae treated with 1.0% (*v*/*v*) strain B1 supernatant for 0, 3, 5 days (Fig. 5). On the basis of FESEM observations, treated cells showed different degrees of damage in terms of morphological characteristics when compared with the normal and plump cells

Fig. 3. Malondialdehyde (MDA) content produced by 4 algal species (*Prorocentrum donghaiense*, *Skeletonema costatum*, *Phaeocystis globosa*, and *Heterosigma akashiwo*) after exposure to strain B1 supernatant. Error bars correspond to the standard deviation. *, statistical significance at $p < 0.05$; **, statistical significance at $p < 0.01$.



in control groups (Figs. 5*a*, 5*d*, 5*g*, and 5*j*). After 3 days, different degrees of deformation appeared, with the cells of *S. costatum* taking on several broken junctions between cells and the chains of cells, while the cell surface of *H. akashiwo*, *P. globosa*, and *P. donghaiense* appeared as a melting and tortile phenomenon (Figs. 5*b*, 5*e*, 5*h*, and 5*k*). The cell membrane was severely deformed and split, and the cells were even broken with their contents leaking out after 5 days exposure. Ultimately, the majority of the cells was completely cracking (Figs. 5*c*, 5*f*, 5*i*, and 5*l*); in particular, the cells of *P. globosa* were completely broken into pieces under visual fields of microscope.

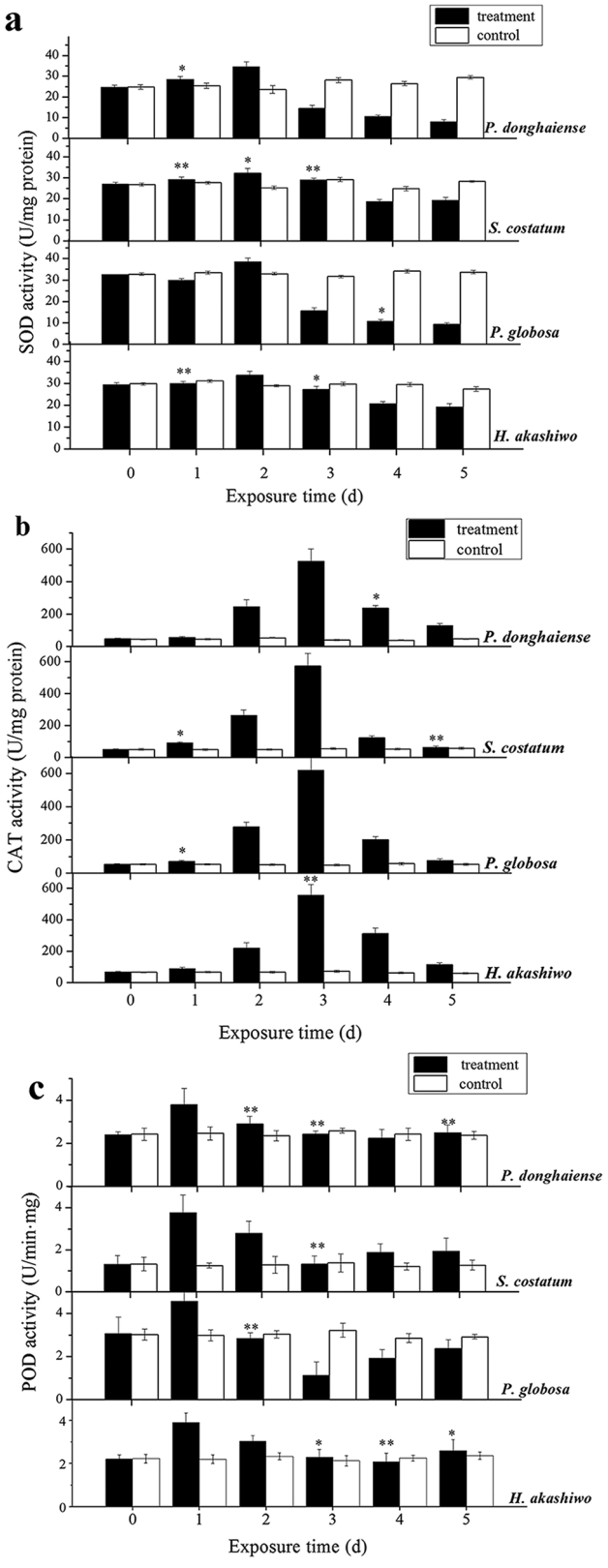
Effects of photosynthesis-related genes

Figure 6 shows the effects of the different supernatant concentrations of strain B1 on the 3 photosynthesis-related gene expressions in *H. akashiwo*, *S. costatum*, and *P. globosa*. The abundances of all genes were significantly inhibited by the B1 supernatant. After 96 h exposure of the 1.0% (*v*/*v*) supernatant, the expression of *psbA*, *psbD*, *rbcL*, respectively, decreased to 43.0% ($p < 0.05$), 31.7% ($p < 0.01$), 44.1% ($p < 0.05$) for *H. akashiwo*, to 2.02% ($p < 0.01$), 4.10% ($p < 0.05$), and 12.6% ($p < 0.01$) for *S. costatum*, and to 28.5% ($p < 0.01$), 74.2% ($p < 0.05$), 32.0% ($p < 0.05$) for *P. globosa* compared with the controls. As for the 2.0% (*v*/*v*) treatment groups, gene expression of the 3 algal species were significantly decreased compared with the controls. Obviously, expression of *psbA*, *psbD*, *rbcL* by *H. akashiwo* decreased to 14.3%, 3.08% ($p < 0.01$), and 11.6%, respectively, of the controls; *S. costatum* to 1.04% ($p < 0.01$), 2.21% ($p < 0.01$), and 10.7% ($p < 0.01$), respectively, of the controls; and *P. globosa* to 8.39% ($p < 0.01$), 24.6% ($p < 0.01$), and 30.2% ($p < 0.05$), respectively, of the controls. *Skeletonema costatum* expression of the 3 photosynthetic genes fell to very low levels regardless of the treatment concentration, and gene expression by all treatment groups decreased significantly compared with control groups at different concentrations.

Discussion

Many marine bacteria have been found to be algicidal, most have been found to lyse only one kind of algae (Kim et al. 2008). Furthermore, few studies have reported the algicidal mechanism of a *Bacillus* on different kinds of harmful algae (Tilney et al. 2014). In the present study, strain B1 showed algicidal activity on *P. globosa*, *S. costatum*, *H. akashiwo*, and *P. donghaiense* (Fig. 2), which belong to the *Pelagophyta*, *Bacillariophyta*, *Raphidophyta*, and *Dinophyta*, respectively, suggesting that strain B1 has a relatively wide host range. It is reported that algicidal bacteria and active metabolites are specific to algal species (Zhao et al. 2014), but strain B1 had an algicidal effect on 4 kinds of algae because it excretes specific and complex

Fig. 4. The activities of (*a*) superoxide dismutase (SOD), (*b*) catalase (CAT), and (*c*) peroxidase (POD) in 4 algal cells of *Prorocentrum donghaiense*, *Skeletonema costatum*, *Phaeocystis globosa*, and *Heterosigma akashiwo* under strain B1 supernatant. All error bars correspond to the standard deviation. *, statistical significance at $p < 0.05$; **, statistical significance at $p < 0.01$.

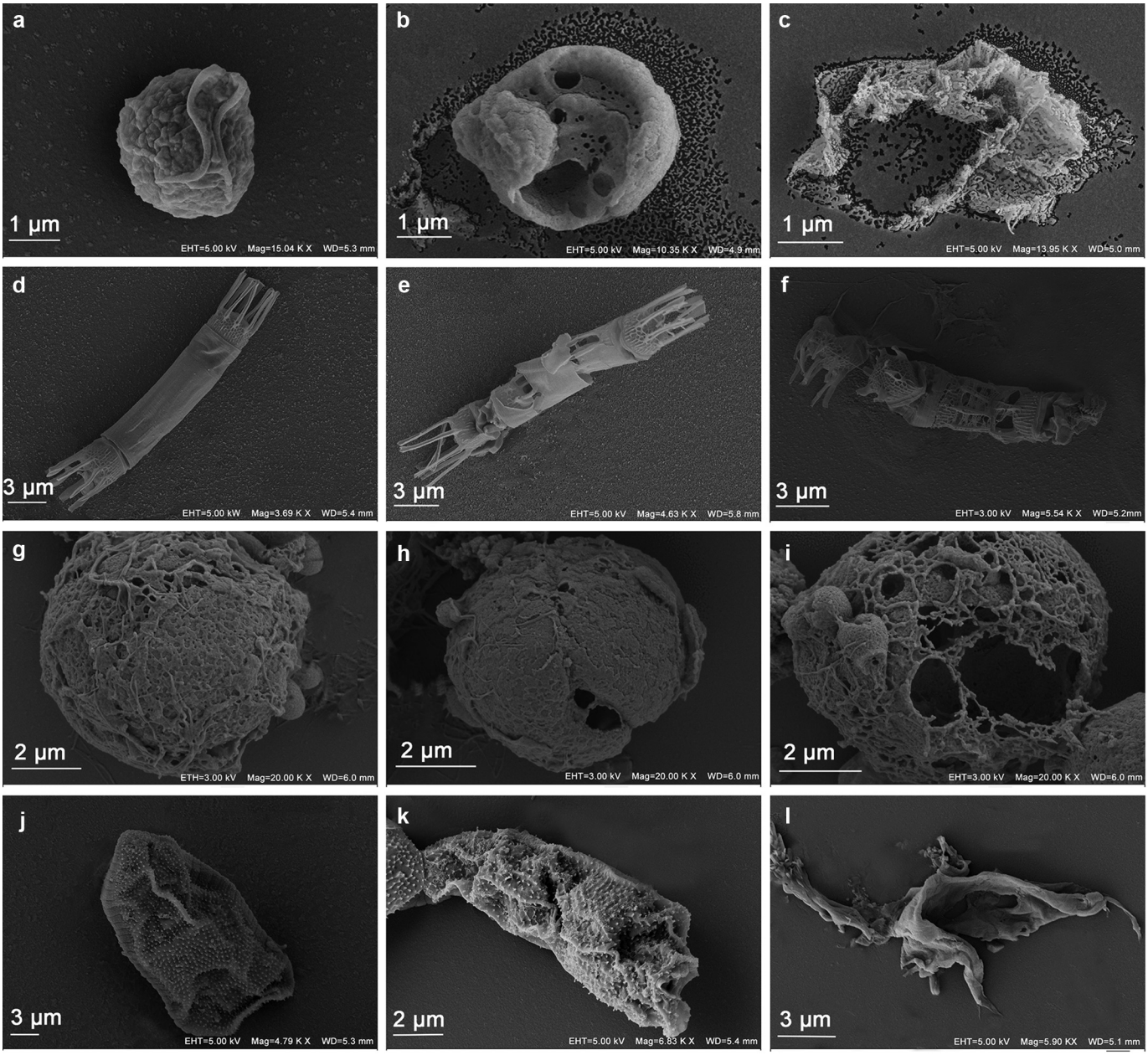


effective metabolites, such as L-histidine, *o*-tyrosine, *N*-acetylhistamine, and urocanic acid. L-Histidine is only toxic to *P. globosa*; however, *o*-tyrosine, *N*-acetylhistamine, and urocanic acid inhibit the growth of *P. donghaiense*, *H. akashiwo*, and *S. costatum* (Zhao et al. 2014).

The physiological response, including MDA, SOD, CAT, and POD, was implemented to reveal the oxidative damage. Especially, the MDA is an immediate index of the lipid peroxidation inside the cells. The significant elevation in MDA contents (Fig. 3) manifested the existence of serious oxidation on the 4 algal species. Zhang et al. (2013*b*) also found that *Brevibacterium* sp. strain BS01 culture supernatant could make a significant increase in MDA contents when treated on *A. tamarense*. When the MDA increased in the first 4 days, the MDA could stimulate the algal cell proliferation (Zhao et al. 2012), and the increasing number of algal cells could cause more MDA than before. However, the MDA level of *H. akashiwo* and *P. globosa* treatment groups decreased after reaching the maximum level, which might have caused irreversible oxidative damage to the cells. Conclusions also have been reported that the MDA contents increased acutely to the toxic level when exposed to L7-LPEALP, in which algal growth was inhibited and the cellular membrane system was damaged (Thomas and Wofford 1993; Zhao et al. 2012).

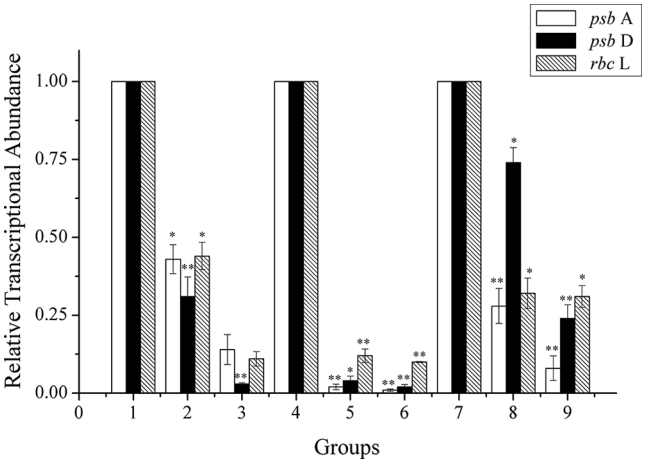
SOD, CAT, and POD are important antioxidases in cells; they protect organisms against damages caused by oxygen-free radicals and harmful substances (Luo et al. 2013). The mechanism by which algal cells could be killed was indirectly reflected through the variation of these biochemical indexes under strain B1 supernatant stress. The elevation of SOD and CAT activities meant that the algal cells started their antioxidant activity system to dispose the oxidation of external stresses (Fig. 4); an analogous result has been reported by Wang et al. (2012). The increased SOD activity in the first 3 days and the decreased activity afterwards suggested that algae was controlled effectively, and even killed with long-term exposure to the supernatant. The SOD activities of *P. donghaiense* and *P. globosa* responded more severely than did those of *H. akashiwo* and *S. costatum*, as indicated by the differences in sensitivity to supernatant. However, the enzyme activities of the 4 species had a similar trend. CAT, one of the key enzymes of the defense system of organism, can make hydrogen peroxide into oxygen and water, thereby preventing cell damage by hydrogen peroxide. The fiercest defense reaction of CAT appeared at the very start and subsequently collapsed when exposed to the supernatant of strain B1 (Fig. 4*b*), which was consistent with previous studies (Sun et al. 2004). It could be concluded from the almost synchronous CAT activities of the 4 algae species that the activity of CAT was mainly affected by active substances; however, there

Fig. 5. Field emission scanning electron microscopy micrographs of algal cells after strain B1 supernatant treatment. (*a–c*) *Phaeocystis globosa*, (*d–f*) *Skeletonema costatum*, (*g–i*) *Heterosigma akashiwo*, and (*j–l*) *Prorocentrum donghaiense* at 0, 3, 5 days, respectively.



were no obvious differences among these algae species. The decline in SOD and CAT activities both contributed to an accumulation of intracellular ROS, and high levels of ROS increase the MDA content and the lipid peroxidation of algal cells (Zhao et al. 2014). The increase in the POD activities of treatment groups suggests that resistance to potential oxidative damage by strain B1 supernatant has already begun (Fig. 4*c*), because normal algal cells will not accumulate POD. Contrary to the SOD and CAT activities, the POD activities decreased down to the lowest and then increased (Fig. 4*c*). The POD activity of *S. costatum* first showed a maximum ratio of the treatment group to the control group, implying that *S. costatum* had timely induction ability, and this was also reflected by the algicidal activity (Fig. 2) and electron microscopy images (Fig. 5*e*). What is noticed here in the present research was that the effects of oxidation stress induced by strain B1 supernatant on *P. globosa* were always obvious. It indicated that *P. globosa* has a more sensitive response to the external stresses than do the other species. According to the results of MDA, SOD, CAT, and POD activities, enzymes were seen to be directly involved in resisting supernatant stress in algal cells, and this conclusion was in agreement with previous studies (Zhang et al. 2014). By comparison, although there were significant differences on enzyme activities between the 4 kinds of algae, the

Fig. 6. Relative transcriptional levels of strain B1 supernatant on *Heterosigma akashiwo* (groups 1–3), *Skeletonema costatum* (groups 4–6), and *Phaeocystis globosa* (groups 7–9) that was exposed to different concentrations (0.0%, 1.0%, and 2.0%, in that order and respectively) for 96 h. All error bars correspond to the standard deviation. *, statistical significance at $p < 0.05$; **, statistical significance at $p < 0.01$.



change trend of the cell antioxidant system was similar under the oxidative stress.

To explore the effect of strain B1 supernatant on the photosynthetic system, causing the oxidative damage, Asada et al. (1998) measured the expression of the photosynthesis-related genes *psbA*, *psbD*, and *rbcL*. Active substances might affect normal electron transport to produce surplus electrons in the photosynthetic process, and transcription of electronic transmission chain related genes (*psbA*, *psbD*) could indicate plant photosynthetic oxidation damage through the consumption of oxygen and formation of ROS (Zhang et al. 2013*b*). The effects of strain B1 supernatant on photosynthetic gene expression were strong and greatly influenced the electronic transmission chain to cause oxidative injury (Fig. 6). Regardless if algal cells were exposed to 1.0% (*v*/*v*) or 2.0% (*v*/*v*) strain B1 supernatant, all genes expressions were significantly decreased, especially *psbA* gene expression by *S. costatum*. It has been previously reported that genes *psbA* and *psbD* genes had different responses to BS01 supernatant, but the mechanism is still not clear (Zhang et al. 2013*b*). *Phaeocystis globosa* responded slowly to B1 supernatant, as indicted by the obvious lack of algicidal activity on the first day (Fig. 2*a*) and insignificant gene expression in neither the 1% (*v*/*v*) nor 2% (*v*/*v*) treatment groups (Fig. 6), which can therefore be interpreted as *P. globosa*'s higher tolerance and its antioxidant system's ability. The decrease in photosynthesis-related genes revealed that they affected electron transport and generation of ROS (Zhang et al. 2013*b*). Electron transfer starts from photosystem II and then O_2 accepts electrons to become $\bullet O^{2-}$ (superoxide anion). Only the presence of SOD in the chloroplast can eliminate the $\bullet O_2$ to generate H_2O_2, far more important is both the $\bullet O_2$ and H_2O_2 in the process belonging to reactive oxygen molecules (ROS, Mehler's reaction) (Jia et al. 2000); however, the activity of SOD was gradually decreased during the experiment. Gene expression levels of different algal species have different responses, which help to further understand the mechanism of active substances and promote the realistic feasibility.

The cell membrane acts as a barrier to prevent extracellular active substances of strain B1 from passing freely through algal cells, thereby ensuring a relatively stable environment within the cells, so that the orderly operation of the various biochemical reactions can be implemented (Veldhuis et al. 2001; Li et al. 2014). On the basis of the degree of damage to the cells (Fig. 5), different sensitivities of the algal species to strain B1 supernatant can be seen. At the initial stage of the reaction, cell morphological damage of *S. costatum* was obvious, and a significant change was also shown in the algicidal activity, CAT and POD activities, and gene expression. These effects may be because the *S. costatum* filtrates had an autoinhibitory allelopathic effect on the growth phase of the species (Wang et al. 2013), and the effects of B1 supernatant and autoinhibitory allelopathic could combine to strengthen the algicidal effect. The morphological changes of *P. globosa* cells reacted slowly in the beginning, probably because it has a capsule body that prevents the cells from damage (Huang et al. 2007). When the cells began to have morphological damage, the intracellular MDA content, enzyme activity, and gene expression almost synchronously changed. The change was also in accordance with the decrease in gene expression. Similar results were previously reported with *M. aeruginosa* exposure to neopzewaquinone A (Zhang et al. 2013*a*). Therefore, it can be inferred that various changes in algal cells influenced each other after adding strain B1 supernatant, since enzymes changed significantly with the increase of MDA content, leading to the suppression of genes expression.

In summary, strain B1 supernatant could inhibit the growth of the 4 kinds of algae. Different algae have different sensitivities, including the change of algicidal activity and MDA contents, decrease in enzymes activities, damage of cell morphology, and expression of genes. *Phaeocystis globosa* algae had the highest tolerance to the algicidal substances, while *S. costatum* algae were more sensitive to strain B1 supernatant in the first day. Comparing the differences among the 4 kinds of algae may provide the potential inhibition mechanism. After understanding the algicidal procedure, it will help to control HABs with marine algicidal bacteria. However, further investigations are needed before practical application, such as tracking the active metabolites in the algicidal process.

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References

Amaro, A.M., Fuentes, M.S., Ogalde, S.R., Venegas, J.A., and Suárez-Isla, B.A. 2005. Identification and characterization of potentially algal-lytic marine bacteria strongly associated with the toxic dinoflagellate *Alexandrium catenella*. J. Eukaryot. Microbiol. **52**(3): 191–200. doi:10.1111/j.1550-7408.2005.00031.x. PMID:15926994.

Anderson, D.M. 1997. Turning back the harmful red tide. Nature, **388**(6642): 513–514. doi:10.1038/41415.

Anderson, D.M. 2009. Approaches to monitoring, control and management of harmful algal blooms (HABs). Ocean Coast. Manag. **52**(7): 342–347. doi:10.1016/j.ocecoaman.2009.04.006. PMID:20161650.

Asada, K., Endo, T., Mano, J., and Miyake, C. 1998. Molecular mechanism for relaxation of and protection from light stress. *In* Stress responses of photosynthetic organisms. *Edited by* K. Sato and N. Murata. Elsevier, Amsterdam, Netherlands. pp. 37–52.

Beauchamp, C., and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. **44**(1): 276–287. doi:10.1016/0003-2697(71)90370-8. PMID:4943714.

Briand, E., Gugger, M., François, J.-C., Bernard, C., Humbert, J.-F., and Quiblier, C. 2008. Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming *Planktothrix agardhii* (*cyanobacterium*) population. Appl. Environ. Microbiol. **74**(12): 3839–3848. doi:10.1128/AEM.02343-07. PMID:18441113.

Brussaard, C.P.D., Bratbak, G., Baudoux, A.C., and Ruardij, P. 2007. *Phaeocystis* and its interaction with viruses. Biogeochemistry, **83**(1–3): 201–215. doi:10.1007/s10533-007-9096-0.

Cakmak, I., and Marschner, H. 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol. **98**: 1222–1227. doi:10.1104/pp.98.4.1222. PMID:16668779.

Civello, P.M., Martinez, G.A., Chaves, A.R., and Anon, M.C. 1995. Peroxidase from strawberry fruit (*Fragaria ananassa* Duch.): partial purification and determination of some properties. J. Agric. Food Chem. **43**(10): 2596–2601. doi:10.1021/jf00058a008.

Costas, E., and Lopez-Rodas, V. 2006. Copper sulphate and DCMU-herbicide treatments increase asymmetry between sister cells in the toxic cyanobacteria *Microcystis aeruginosa*: implications for detecting environmental stress. Water Res. **40**(12): 2447–2451. doi:10.1016/j.watres.2006.04.013. PMID:16762387.

Elbaz, A., Wei, Y.Y., Meng, Q.A., Zheng, Q., and Yang, Z.M. 2010. Mercury-induced oxidative stress and impact on antioxidant enzymes in *Chlamydomonas reinhardtii*. Ecotoxicology, **19**(7): 1285–1293. doi:10.1007/s10646-010-0514-z. PMID:20571879.

Furusawa, G., Yoshikawa, T., Yasuda, A., and Sakata, T. 2003. Algicidal activity and gliding motility of *Saprospira* sp. SS98-5. Can. J. Microbiol. **49**(2): 92–100. doi:10.1139/w03-017. PMID:12718397.

Graham, S.L., and Strom, S.L. 2010. Growth and grazing of microzooplankton in response to the harmful alga *Heterosigma akashiwo* in prey mixtures. Aquat. Microb. Ecol. **59**(2): 111–124. doi:10.3354/ame01391.

Hallegraeff, G.M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia, **32**(2): 79–99. doi:10.2216/i0031-8884-32-2-79.1.

Hoshaw, R., and Rosowski, J.1973. Methods for microscopic algae. *In* Handbook of phycological methods: culture methods and growth measurements. *Edited by* J.R. Stein. Cambridge University Press, UK. pp. 53–68.

Huang, Y.F., Feng, J.H., Jiang, S., Wei, P., Feng, J.P., and Bai, J. 2007. Annual dynamics of *Skeletonema costatum* and its relation to environmental factors in Guangzhou Sea Area. Ecol. Sci. **26**(1): 50–54.

Jeong, H.J., Kim, J.S., Du Yoo, Y., Kim, S.T., Song, J.Y., Kim, T.H., et al. 2008. Control of the harmful alga *Cochlodinium polykrikoides* by the naked ciliate *Strombidinopsis jeokjo* in mesocosm enclosures. Harmful Algae, **7**(3): 368–377. doi:10.1016/j.hal.2007.12.004.

Jia, H., Li, D., and Han, Y. 2000. Advances in studies on photoinhibition in photosynthesis of higher plants. Chin. Bull. Bot. **17**(3): 218–224.

Kim, J.-D., Kim, J.-Y., Park, J.-K., and Lee, C.-G. 2009. Selective control of the *Prorocentrum minimum* harmful algal blooms by a novel algal-lytic bacterium *Pseudoalteromonas haloplanktis* AFMB-008041. Mar. Biotechnol. **11**(4): 463–472. doi:10.1007/s10126-008-9167-9. PMID:19048341.

Kim, M.J., Jeong, S.Y., and Lee, S.J. 2008. Isolation, identification, and algicidal activity of marine bacteria against *Cochlodinium polykrikoides*. J. Appl. Phycol. **20**(6): 1069–1078. doi:10.1007/s10811-008-9312-x.

Kumar, S., Habib, K., and Fatma, T. 2008. Endosulfan induced biochemical changes in nitrogen-fixing cyanobacteria. Sci. Total Environ. **403**(1–3): 130–138. doi:10.1016/j.scitotenv.2008.05.026. PMID:18584851.

Li, Q. 2012. Isolation and identification of algicidal bacteria and characteristics study of algicidal bacteria on *Phaeocystis globosa*. Dissertation, Jinan University. [In Chinese.]

Li, Y., Zhu, H., Zhang, H.J., Chen, Z.R., Tian, Y., Xu, H., et al. 2014. Toxicity of algicidal extracts from *Mangrovimonas yunxiaonensis* strain LY01 on a HAB causing *Alexandrium tamarense*. J. Hazard. Mater. **278**: 372–381. doi:10.1016/j.jhazmat.2014.06.032. PMID:24997253.

Li, Z.H., Geng, M.X., and Yang, H. 2015. Algicidal activity of *Bacillus* sp. Lzh-5 and its algicidal compounds against *Microcystis aeruginosa*. Appl. Microbiol. Biotechnol. **99**(2): 981–990. doi:10.1007/s00253-014-6043-6. PMID:25196270.

Liao, C.L., and Liu, X.B. 2014. High-cell-density cultivation and algicidal activity assays of a novel algicidal bacterium to control algal bloom caused by water eutrophication. Water Air Soil Pollut. **225**: 2120. doi:10.1007/s11270-014-2120-9.

Luo, J.F., Wang, Y., Tang, S.S., Liang, J.W., Lin, W.T., and Luo, L.X. 2013. Isolation and identification of algicidal compound from *Streptomyces* and algicidal mechanism to *Microcystis aeruginosa*. PLoS One, **8**(10): e76444. doi:10.1371/journal.pone.0076444. PMID:24098501.

Marder, J., Chapman, D., Telfer, A., Nixon, P., and Barber, J. 1987. Identification of *psbA* and *psbD* gene products, D1 and D2, as reaction centre proteins of photosystem 2. Plant Mol. Biol. **9**(4): 325–333. doi:10.1007/BF00014907. PMID:24277085.

Mayali, X., and Azam, F. 2004. Algicidal bacteria in the sea and their impact on algal blooms. J. Eukaryot. Microbiol. **51**(2): 139–144. doi:10.1111/j.1550-7408.2004.tb00538.x. PMID:15134248.

Mohamed, Z.A., Hashem, M., and Alamri, S.A. 2014. Growth inhibition of the cyanobacterium *Microcystis aeruginosa* and degradation of its microcystin toxins by the fungus *Trichoderma citrinoviride*. Toxicon, **86**: 51–58. doi:10.1016/j.toxicon.2014.05.008. PMID:24874888.

Nakashima, T., Miyazaki, Y., Matsuyama, Y., Muraoka, W., Yamaguchi, K., and Oda, T. 2006. Producing mechanism of an algicidal compound against red tide phytoplankton in a marine bacterium γ-*proteobacterium*. Appl. Microbiol. Biotechnol. **73**(3): 684–690. doi:10.1007/s00253-006-0507-2. PMID:16850298.

Pierce, R.H., Henry, M.S., Higham, C.J., Blum, P., Sengco, M.R.,

and Anderson, D.M. 2004. Removal of harmful algal cells (*Karenia brevis*) and toxins from seawater culture by clay flocculation. Harmful Algae, **3**(2): 141–148. doi:10.1016/j.hal.2003.09.003.

Shi, S.Y., Tang, D.S., and Liu, Y.D. 2009. Effects of an algicidal bacterium *Pseudomonas mendocina* on the growth and antioxidant system of *Aphanizomenon flos-aquae*. Curr. Microbiol. **59**(2): 107–112. doi:10.1007/s00284-009-9404-0. PMID:19365689.

Shiu, C.T., and Lee, T.M. 2005. Ultraviolet-B-induced oxidative stress and responses of the ascorbate–glutathione cycle in a marine macroalga *Ulva fasciata*. J. Exp. Bot. **56**(421): 2851–2865. doi:10.1093/jxb/eri277. PMID:16157654.

Su, R.Q., Yang, X.R., Zheng, T.L., Tian, Y., Jiao, N.Z., Cai, L.Z., and Hong, H.S. 2007. Isolation and characterization of a marine algicidal bacterium against the toxic dinoflagellate *Alexandrium tamarense*. Harmful Algae, **6**(6): 799–810. doi:10.1016/j.hal.2007.04.004.

Sun, X.-X., Choi, J.-K., and Kim, E.-K. 2004. A preliminary study on the mechanism of harmful algal bloom mitigation by use of sophorolipid treatment. J. Exp. Mar. Biol. Ecol. **304**(1): 35–49. doi:10.1016/j.jembe.2003.11.020.

Thomas, P., and Wofford, H.W. 1993. Effects of cadmium and Aroclor 1254 on lipid peroxidation, glutathione peroxidase activity, and selected antioxidants in *Atlantic croaker* tissues. Aquat. Toxicol. **27**(1): 159–177. doi:10.1016/0166-445X(93)90052-3.

Tilney, C.L., Pokrzywinski, K.L., Coyne, K.J., and Warner, M.E. 2014. Growth, death, and photobiology of dinoflagellates (*Dinophyceae*) under bacterial-algicide control. J. Appl. Phycol. **26**(5): 2117–2127. doi:10.1007/s10811-014-0248-z.

Veldhuis, M.J.W., Kraay, G.W., and Timmermans, K.R. 2001. Cell death in phytoplankton: correlation between changes in membrane permeability, photosynthetic activity, pigmentation and growth. Eur. J. Phycol. **36**(2): 167–177. doi:10.1080/09670260110001735318.

Wang, B.X., Yang, X.R., Lu, J.L., Zhou, Y.Y., Su, J.Q., Tian, Y., et al. 2012. A marine bacterium producing protein with algicidal activity against *Alexandrium tamarense*. Harmful Algae, **13**: 83–88. doi:10.1016/j.hal.2011.10.006.

Wang, J.T., Zhang, Y.W., Li, H., and Cao, J. 2013. Competitive interaction between diatom *Skeletonema costatum* and dinoflagellate *Prorocentrum donghaiense* in laboratory culture. J. Plankton Res. **35**(2): 367–378. doi:10.1093/plankt/fbs098.

Wang, X.L., Gong, L.Y., Liang, S.K., Han, X.R., Zhu, C.J., and Li, Y.B. 2005. Algicidal activity of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa*. Harmful Algae, **4**(2): 433–443. doi:10.1016/j.hal.2004.06.001.

Yang, Y.F., Hu, X.J., Zhang, J., and Gong, Y.X. 2013. Community level physiological study of algicidal bacteria in the phycospheres of *Skeletonema costatum* and *Scrippsiella trochoidea*. Harmful Algae, **28**: 88–96. doi:10.1016/j.hal.2013.05.015.

Zhang, C., Yi, Y.L., Hao, K., Liu, G.L., and Wang, G.X. 2013*a*. Algicidal activity of *Salvia miltiorrhiza* Bung on *Microcystis aeruginosa* — towards identification of algicidal substance and determination of inhibition mechanism. Chemosphere, **93**(6): 997–1004. doi:10.1016/j.chemosphere.2013.05.068. PMID:23810520.

Zhang, H., An, X., Zhou, Y., Zhang, B., Zhang, S., Li, S., et al. 2013*b*. Effect of oxidative stress induced by *Brevibacterium* sp. BS01 on a HAB causing species-*Alexandrium tamarense*. PLoS One, **8**(5): e63018. doi:10.1371/journal.pone.0063018. PMID:23667564.

Zhang, H.J., Lv, J.L., Peng, Y., Zhang, S., An, X.L., Xu, H., et al. 2014. Cell death in a harmful algal bloom causing species *Alexandrium tamarense* upon an algicidal bacterium induction. Appl. Microbiol. Biotechnol. **98**(18): 7949–7958. doi:10.1007/s00253-014-5886-1. PMID:24962118.

Zhao, L., Chen, L.N., and Yin, P.H. 2014. Algicidal metabolites produced by *Bacillus* sp. strain B1 against *Phaeocystis globosa*. J. Ind. Microbiol. Biotechnol. **41**(3): 593–599. doi:10.1007/s10295-013-1393-0. PMID:24370882.

Zhao, S., Pan, W.B., and Ma, C. 2012. Stimulation and inhibition effects of algae-lytic products from *Bacillus cereus* strain L7 on *Anabaena flos-aquae*. J. Appl. Phycol. **24**(5): 1015–1021. doi:10.1007/s10811-011-9725-9.

Zheng, X.W., Zhang, B.Z., Zhang, J.L., Huang, L.P., Lin, J., Li, X.Y., et al. 2013. A marine algicidal actinomycete and its active substance against the harmful algal bloom species *Phaeocystis globosa*. Appl. Microbiol. Biotechnol. **97**(20): 9207–9215. doi:10.1007/s00253-012-4617-8. PMID:23224407.