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A marine algicidal actinomycete and its active substance against the harmful algal bloom species *Phaeocystis globosa*

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Abstract A strain O4-6, which had pronounced algicidal effects to the harmful algal bloom causing alga *Phaeocystis globosa*, was isolated from mangrove sediments in the Yunxiao Mangrove National Nature Reserve, Fujian, China. Based on the 16S rRNA gene sequence and morphological characteristics, the isolate was found to be phylogenetically related to the genus *Streptomyces* and identified as *Streptomyces malaysiensis* O4-6. Heat stability, pH tolerance, molecular weight range and aqueous solubility were tested to characterize the algicidal compound secreted from O4-6. Results showed that the algicidal activity of this compound was not heat stable and not affected by pH changes. Residue extracted from the supernatant of O4-6 fermentation broth by ethyl acetate, was purified by Sephadex LH-20 column and silica gel column chromatography before further structure determination. Chemical structure of the responsible compound, named NIG355, was illustrated based on quadrupole time-of-flight mass spectrometry (Q-TOF-MS) and nuclear magnetic resonance (NMR) spectra. And this compound showed a stronger algicidal activity compared with other reported algicides. Furthermore, this article represents the first report of an algicide against *P. globosa*, and the compound may be potentially used as a bio-agent for controlling harmful algal blooms.

Keywords Algicidal actinomycete · Algicidal compound · Harmful algal bloom · *Phaeocystis globosa*

Introduction

As one of the most important primary producers in the marine ecosystem, algae can support microorganisms, zooplankton, and other higher life forms by releasing a number of extracellular products (Camacho et al. 2007), such as carbohydrates, amino acids and peptides, sugars, polyalcohols, and vitamins, all of which are important sources of marine organic material (Hellebust 1965), into the marine environment. However, toxic algae, which has been identified in more than 60 marine species (Pierce and Kirkpatrick 2001), threaten the survival of other organisms once they have accumulated in sufficient numbers.

Harmful algal blooms (HABs), which are mostly caused by toxic algae, have been recognized as one of the main ecological problems in coastal regions worldwide since the 1970s (Masó and Garcés 2006) due to their production of endogenous toxins, sheer biomass, and physical shape (Glibert et al. 2005). They are responsible not only for public health problems but also for substantial economic

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losses (Blauw et al. 2010), with the economic loss from HAB-related events in the United States having been estimated to be worth several billions of U.S. dollars annually (Schrader et al. 2003).

Numerous studies have been carried out to develop strategies for controlling HABs. These strategies can be summarized as physical methods (e.g., clays and flocculants) (Pan et al. 2006; Pierce et al. 2004; Sengco et al. 2001, 2005), chemical methods (e.g., copper sulfate, surfactants, and sodium hypochlorite) (Baek et al. 2003; Jeong et al. 2002; Sun et al. 2004), and biological methods (e.g., algicidal bacteria, algicidal viruses, and plankton grazers) (Hare et al. 2005; Kim et al. 2009; Mitra and Flynn 2006). However, the use of physical and chemical methods in aquatic ecosystems is potentially dangerous; their high cost, secondary pollution effects, and impracticability also limit their application (Anderson 1997). Therefore, biological methods have received particular interest in recent years because of their potential effectiveness, species specificity, and eco-friendliness, although using bio-resources still has many logistic problems and is far from the application stage (Anderson 2009). Research into algal–bacterial interactions has resulted in the isolation of several strains of bacteria capable of inhibiting or killing HAB species (Kim et al. 2008; Mayali and Azam 2004; Park et al. 2010; Su et al. 2007). In addition, most algicidal bacteria are specific to algal species, making their use to control HABs an environment-friendly approach.

In this study, an actinomycete strain with pronounced algicidal effects on *Phaeocystis globosa* was isolated from mangrove sediments in Yunxiao Mangrove National Nature Reserve, Fujian Province, China (117°24′–117°30′E, 23°53′–23°56′N). The heat stability, pH tolerance, molecular weight range, and aqueous solubility of the algicidal compound were determined. It was purified after extraction, and its chemical structure was further defined using quadrupole time-of-flight mass spectrometry (Q-TOF-MS) and nuclear magnetic resonance (NMR).

Materials and methods

Algal cultures

Algal cultures of *P. globosa* PG03 and *Chlorella autotrophica* CA01 were obtained from the State Key Laboratory of Marine Environmental Science, Xiamen University, China. *Alexandrium tamarense* ATGD98-006 was supplied by the Algal Culture Collection, Institute of Hydrobiology, Jinan University (Guangzhou, China). All cultures were maintained in f/2 medium (Guillard 1975) (prepared with 0.45 μm of filtered seawater) at 20±1 °C under a 12-h light/12-h dark cycle with a light intensity of 50 μmol photons $m^{-2}s^{-1}$.

Isolation and screening of algicidal strain against host algae

Samples were collected from mangrove surface sediments (0–20 cm) in Yunxiao Mangrove National Nature Reserve (117°24′–117°30′E, 23°53′–23°56′N) in October 2009. For bacterial isolation, 10 g of dried sediments was inoculated into 90 ml of sterilized seawater and incubated in a shaker for 1 h at 150 rpm, followed by a 10-fold serial dilution. An aliquot of each dilution (100 μl) was spread onto AC1 solid medium (20 g of soluble starch, 1 g of $NaNO_3$, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $FeSO_4{\cdot}7H_2O$, 75 μg of $K_2Cr_2O_7$, 10 g of agar in 1 l of 0.45 μm of Millipore-filtered seawater). The resulting plates were incubated at 28 °C for 7 days, after which individual colonies were picked from the plates and restreaked at least twice on the same medium to ensure purity (Atkinson et al. 2008). Individual colonies were isolated based on their size, morphology and color, and those that were difficult to distinguish were considered as specific ones to obtain as more isolates as possible. Isolates were grown in 4 ml of AC1 liquid medium (no $K_2Cr_2O_7$) at 28 °C and 150 rpm for 7 days. A 0.4-ml aliquot of each isolate supernatant, which was collected by centrifugation at 5,000×*g* for 20 min, was inoculated in triplicate into 20 ml of logarithmic-phase *P. globosa* cultures, and a 0.4-ml aliquot of AC1 liquid medium only was added to the algal cultures as a control. After incubation for 24 h at 20±1 °C under a 12-h light/12-h dark cycle with a light intensity of 50 μmol photons $m^{-2}s^{-1}$, algal cultures were added to a 24-well plate (2 ml each well) to monitor the growth of *P. globosa* by measuring the fluorescence density at an excitation wavelength of 467 nm and an emission wavelength of 681 nm (Cai et al. 2008; Yin et al. 2006). Algicidal activity was calculated using the formula described in the next subsection and confirmed by cell lysis by microscopic observation. Strains that showed high algicidal activity (>75 %) were further analyzed.

Analysis of algicidal activity

An algicidal activity test was performed using a 24-well plate to isolate the active substance. Cultures of *P. globosa* in logarithmic phase were added to the plate (2 ml each well). The extracted fractions or purified components dissolved in dimethyl sulfoxide (DMSO), which are discussed below in detail, were added to 2 ml of the *P. globosa* cultures at a concentration of 2 % (v/v) in triplicate. DMSO of the same volume was also added separately as a control. The fluorescence density of *P. globosa* cultures was measured after 24 h of the above-

described treatment. Algicidal activity was calculated as follows (Su et al. 2007):

$$\text{Algicidal Activity}(\%) = [(\text{Flu}_{\text{control well}} - \text{Flu}_{\text{treated well}})/\text{Flu}_{\text{control well}}] \times 100\%,$$

where $\text{Flu}_{\text{control well}}$ and $\text{Flu}_{\text{treated well}}$ are the fluorescence density of *P. globosa* cultures treated with AC1 broth or DMSO and that treated with the fermentation supernatant of isolates or extracted fractions, respectively, to isolate algicidal strains or the active substance.

The data from each experiment were pooled and then evaluated by one-way ANOVA (SPSS 13.0 for Windows).

Characterization and identification of strain O4-6

The strain O4-6 has been deposited in CCTCC (China Center for Type Culture Collection) with the accession number of M2011051. The morphological characteristics of the substrate mycelium, aerial mycelia, and sporophore as well as the spore shape of strain O4-6 were observed with the naked eye and using a scanning electron microscope after cultivation on AC1 plates for 7 days at 28 °C. Temperatures and initial pH ranges for bacterial O4-6 were tested using AC1 broth cultures. The initial pH of the AC1 broth was adjusted to 4, 5, 6, 7, 8, 9, 10, and 11. Measurements of temperature ranges (10 °C, 20 °C, 25 °C, 30 °C, 37 °C, 40 °C, and 45 °C) were completed at a salinity level of 28‰, and those of salinity ranges (5‰, 15‰, 25‰, 35‰, and 40‰) were carried out at 28 °C in a medium similar to AC1 but prepared with distilled water. Conventional biochemical tests were used as described by Kim et al. (2009). Bacterial cells from O4-6 cultures grown in AC1 broth were collected by centrifugation (5,000×*g* for 20 min). Extraction of genomic DNA from O4-6 and PCR amplification for the 16S rRNA gene were performed as described by Su (Su et al. 2007). The PCR products were purified from agarose gel with a GeneClean Turbo Kit (Qbiogene) and ligated with a pMD 19-T vector; the ligation products were transformed into *Escherichia coli* DH5α competent cells, followed by sequencing, which was performed by Shanghai Invitrogen Biotechnology Co., Ltd. The sequence for O4-6 (GenBank accession number HQ845390) was compared with other 16S rRNA genes obtained from GenBank using the BLAST program. Alignments and similarity comparison were calculated by the Clustal X2 software (Thompson et al. 1997), and a phylogenetic tree was constructed using MEGA 5.0 with the neighbor-joining method. Bootstrap values were determined according to the method described by Felsenstein (1985).

Characterization of the algicidal compound

O4-6 cultures grown in AC1 liquid medium at 28 °C for 7 days were centrifuged at 5,000×*g* for 20 min to collect the supernatant. The supernatant was then incubated at 40 °C, 60 °C, 80 °C, 100 °C, and 120 °C for 1 h to test the effects of temperature on algicidal activity. The pH of the supernatant was adjusted to 1–11 (1, 3, 5, 7, 9, and 11) to test the pH tolerance of the algicidal compound. The supernatant was loaded in two dialysis bags with molecular intercept values of 1 kDa and 500 Da and dialyzed in sterilized AC1 liquid medium for 48 h to test the molecular weight range of the algicidal compound; the medium was replaced every 12 h, after which the algicidal activity of the dialyzed supernatant was evaluated after being adjusted to the original volume with the addition of sterile seawater. Each treated supernatant was subsequently inoculated into triplicate *P. globosa* cultures at the final concentration of 2 % (v/v) for the algicidal activity test. Fresh AC1 liquid medium subjected to the same treatments was used as a negative control. The supernatant of O4-6 was mixed with an equal volume of acetonitrile with vigorous shaking in a separation funnel for 5 min. The mixture was allowed to stand for 30 min to collect the organic phase. The extraction was repeated three times. Organic phases were combined and evaporated to dryness and weighted to obtain acetonitrile extracts. Extracts from three other organic solvents (butyl alcohol, dichloromethane, and ethyl acetate) were obtained under the same method. All extracts were dissolved in DMSO (~2 mg ml^{-1}) as a negative control for the algicidal activity test.

Separation and determination of algicidal compound

O4-6 was cultured in AC1 liquid medium (up to 5 l) for 7 days (28 °C, 150 rpm). The culture filtrate was collected by centrifugation at 5,000×*g* for 20 min and then concentrated in a rotavapor. Crude extracts were obtained from the concentrate by extraction three times with an equal volume of ethyl acetate as described above, followed by concentration and drying under reduced pressure in an evaporator at 30 °C. The crude extracts dissolved in ethyl acetate were applied to a Sephadex LH-20 (Amersham Biosciences) column with 100 % methanol as eluent. Active fractions eluted by size gel exclusion chromatography were subjected to silica gel column chromatography (170×30 mm in dimension and with a silica particle size of 200–300 mesh) and eluted with three volume ratios of hexane/ethyl acetate (2:1, 1:1, and 0:1) at a flow rate of 1 $ml\,min^{-1}$. Fractions that exhibited algicidal activity were reloaded on the silica gel column chromatography system (packed in ethyl acetate) with 100 % ethyl acetate as eluent at the flow rate of 1 ml min^{-1}. The purified compound was dissolved in ethyl acetate. The NMR spectra of the compound were recorded in

$CDCl_3$ using a DRX500 instrument (Bruker Biospin, Co., Karlsruhe, Germany) at 25 °C. Trimethylsilyl (TMS) was used as an internal standard. The purified compound was also analyzed by Bio-TOF Q system (Bruker Daltonic Inc., USA). Moreover, the algicidal activity of the purified algicidal substance was compared with that of other reported biological algicides.

Results

Screening of algicidal strains

A total of 521 strains were isolated from mangrove sediment samples in Yunxiao Mangrove National Nature Reserve, 13 of which were observed to have high algicidal effects (>75 %) on the growth of *P. globosa* in preliminary screening. Among these 13 isolates, O4-6 (CCTC accession number M2011051) exhibited the strongest algicidal activity (95.4 %) against *P. globosa* in subsequent screening.

Identification of strain O4-6

The phenotypic profiles of O4-6 are shown in Table 1. The mature sporophore of O4-6 appeared to be oval-shaped with a smooth surface measuring 1.2–1.5×0.7–0.9 μm in size (Fig. 1). O4-6 could grow at temperatures ranging from 20 °C to 40 °C, with an optimum at 37 °C. The strain had a wide pH tolerance (5.0–10.0) and could grow in both freshwater and seawater. Comparison of the characteristics of O4-6 with those of known species of actinomycetes described in *Bergey's Manual of Determinative Bacteriology, Ninth Edition*, suggested that the strain belonged to the genus *Streptomyces*. PCR amplification of the 16S rRNA gene and sequencing also showed that O4-6 (GenBank accession number HQ845390) was most likely affiliated with *Streptomyces*. 16S rRNA gene sequence analysis revealed that O4-6 shared 99 % homology with *Streptomyces malaysiensis* NBRC16446 (AB249918) (Fig. 2).

Table 1 Phenotypic characteristics of strain O4-6

Phenotypic tests	Result	Phenotypic tests	Result
Degradation tests		Growth on sole carbon sources at 1 %w/v	
Casein	+	D-Fructose	+
Starch	+	D-Galactose	+
L-Tyrosine	+	D-Glucose	+
Elastin	+	D-Maltose	+
Gelatin	+	D-Mannitol	+
Xanthine	−	D-Sucrose	+
		D-Lactose	+
Growth at		Starch	+
10 °C	−	CM cellulose (0.1 %,w/v)	−
20–40 °C	+	Sodium acetate (0.1 %,w/v)	−
45 °C	−	Sodium citrate (0.1 %,w/v)	−
pH 4.0	−		
pH 5–10	+	Growth in presence of	
pH 11.0	−	Sodium chloride (5 %, w/v)	+
Salinity 5–40‰	+	Sodium chloride (7 %, w/v)	+
		Sodium chloride (10 %, w/v)	−
Growth on sole nitrogen sources at 0.1 %w/v		Production of	
L-Serine	+	Hydrogen sulphide	−
L-isoleucine	+		
L-Methionine	+		
L-Glutamic acid	+		
L-Phenylalanine	+		

+ positive result or growth, − negative result or no growth

Characterization of the active substance

The supernatant of the O4-6 fermentation broth lost its algicidal activity after being treated at temperatures exceeding 80 °C (Fig. 3). pH variation did not affect the algicidal activity of the supernatant (Table 2). After being dialyzed in two molecular interception dialysis bags (1 kDa and 500 Da), the residue of the supernatant exhibited algicidal activity levels of 21.4±9.6 % and 83.4±5.5 %, respectively, suggesting that the molecular weight of the algicidal compound should range from 500 Da to 1 kDa. Of the four extracts from organic solvents (acetonitrile, butyl alcohol, dichloromethane, and ethyl acetate), ethyl acetate extracts showed the strongest algicidal activity (79.4±4.5 %), whereas acetonitrile extracts demonstrated the weakest. Butyl alcohol and dichloromethane extracts showed almost the same level of algicidal activity, which was slightly lower than that of ethyl acetate extracts by approximately 9 %. The

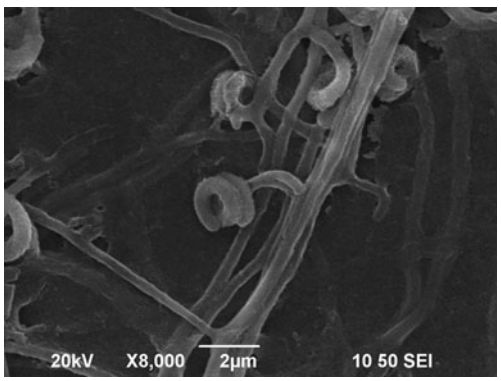

Fig. 1 The morphological characteristics of isolate O4-6 (×8,000, scanning electron microscope)

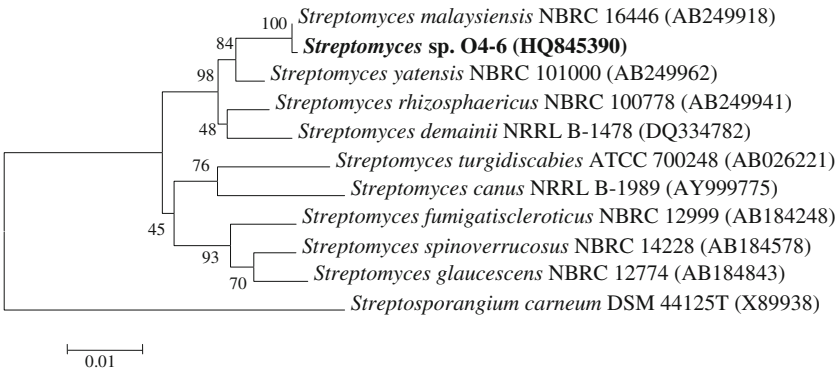

Fig. 2 Phylogenetic tree based on 16S rRNA gene sequence showing the relationship between strain O4-6 with other type strains of actinomycetes. The tree was constructed using the neighbor-joining method using MEGA5.0 program. The boot strap values were evaluated from 1,000 replications

results indicated that the active substance should be more soluble in ethyl acetate.

Extraction and identification of the algicidal compound from O4-6

One major active fraction was obtained after elution with 100 % methanol from a Sephadex LH-20 column. This active fraction was vacuum-concentrated and subjected to silica gel chromatography. Of the four fractions from silica gel chromatography, the hexane/ethyl acetate (1:1) fraction was chosen for further purification due to its algicidal activity. The fraction was collected and applied in a second round of silica gel chromatography with 100 % ethyl acetate as eluent. The eluates with algicidal activity were combined after purification twice by silica gel column chromatography, dissolved in ethyl acetate, named NIG355, and further analyzed by MS and NMR.

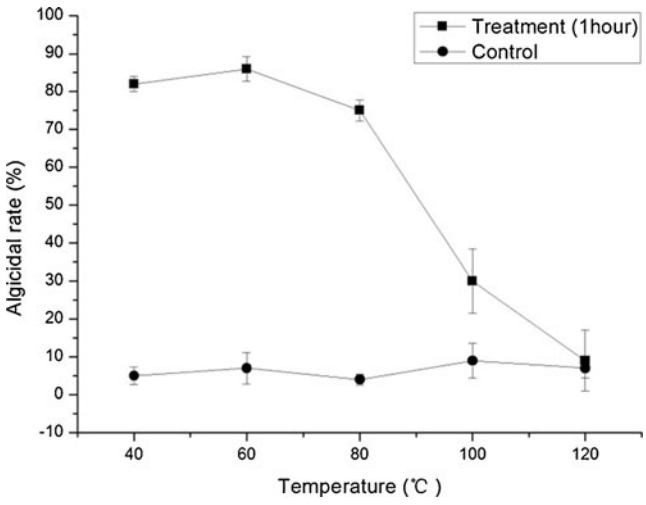

Fig. 3 The effects of temperature on the algicidal activity of supernatant from strain O4-6 cultures. Treatment: supernatants treated in different temperature were subsequently inoculated into triplicate *P. globosa* cultures with a final concentration of 2 % (v/v). Control: fresh AC1 liquid medium subjected to the same treatments

Compound NIG355 was obtained as a colorless powder, and the molecular formula was determined to be $C_{40}H_{68}O_{11}$ via the Q-TOF-MS data (*m/z* 747.4938 for $[M+Na]^+$; Fig. S1), which was confirmed by NMR results. Its ^{13}C NMR (Fig. S2) and DEPT (distortionless enhancement by polarization transfer) showed 40 signals: 10 CH_3 signals [δ(C)=59.53, 29.00, 22.76, 16.98, 16.41, 16.12, 14.38, 13.39, 13.07, and 11.4], including a methoxyl group at δ(C)=59.53 ppm [C(40)]; 10 CH_2 signals [δ(C)=66.88, 41.65, 37.08, 35.77, 32.30, 31.99, 29.47, 26.27, 25.86, and 23.51]; 15 CH signals [δ(C)=85.17, 81.39, 79.40, 76.75, 76.43, 73.20, 68.37, 60.38, 45.79, 39.58, 36.69, 36.40, 35.07, 31.80, and 27.65]; and five quaternary C-atom signals [δ(C)=183.83, 107.49, 97.15, 84.78, and 82.31], including one carboxyl group at δ(C)=183.83 ppm [C(1)] (Table 3). The ^{13}C chemical shift values of NIG355 are consistent with those reported by Xiao and Huang (2002) for an antibiotic M2. The ^1H NMR spectrum (Fig. S3) showed 1 methoxyl group signal at δ(H)=3.64 ppm [H-C(40)], 7 oxygenated methane signals at δ(H)=4.36 ppm [H-C(24)], δ(H)=4.34 ppm [H-C(21)], δ(H)=4.24 ppm [H-C(9)], δ(H)=4.06 ppm [H-C(7)], and so on, as well as several methylene and methyl signals. ^1H NMR (600 MHz, δ ppm from TMS in $CDCl_3$) yielded the following results: δ=4.36 (t, *J*=6.6 Hz, 1H), 4.34 (d, *J*=3.5 Hz, 1H), 4.24 (t, *J*=10.5 Hz, 1H), 4.06 (d, *J*=13.4 Hz, 1H), 3.93 (d, *J*=11.9 Hz, 1H), 3.72–3.66 (m, 3H), 3.64 (s, 3H), 3.37 (s, 1H), 3.33 (d, *J*=11.9 Hz, 1H), 2.59 (t, *J*=12.6 Hz, 1H),

Table 2 The effects of pH on the algicidal activity of supernatant of strain O4-6 cultures

pH	Algicidal activity	
	Supernatant	AC1 medium
1	+	−
3	+	−
5	+	−
7	+	−
9	+	−
11	+	−

+ algicidal activity over 75 %, − algicidal activity lower than 5 %

Table 3 NMR spectroscopic data for "NIG355" and C NMR spectrum of M2[a]

Position	Carbon types	Group	M2[a] δ (C)	Chemical shift of NIG355		
				δ (C)	δ (H)	HMBC
1	C	COOH	183.8	183.8		
2	CH	CH	45.8	45.8	2.30–2.39 (m, 4H)	1, 3, 39
3	CH	CH–O	73.1	73.2	3.66–3.72 (m, 3H)	1, 2, 38
4	CH	CH	29.0	27.7	1.76–1.83 (m, 4H)	
5	CH2	CH_2	26.3	26.3	2.18–2.22 (m, 2H), 1.42–1.46 (m, 3H)	
6	CH2	CH_2	23.5	23.5	1.98–2.04 (m, 3H), 1.09 (d, *J*=12 Hz, 1H)	
7	CH	CH-O	68.3	68.4	4.06(d, *J*=13.4 Hz, 1H)	
8	CH2	CH_2	35.8	35.8	2.59(t, *J*=12.6 Hz, 1H), 0.92–0.93 (m, 4H)	10
9	CH	CH–O	60.4	60.4	4.24(t, *J*=10.5 Hz, 1H)	
10	CH2	CH_2	31.6	32.0	2.30–2.39 (m, 4H), 1.04–1.00 (m, 4H)	
11	CH	CH–O	78.4	79.4	3.37 (s, 1H)	
12	CH	CH	36.5	36.7	1. 76–1. 83 (m, 4H)	13, 14
13	C	O–C–O	107.5	107.5		
14	CH	CH	39.5	39.6	2. 18–2. 22 (m, 2H)	
15	CH2	CH_2	41.7	41.7	1.76–1.83 (m, 4H), 1.64(t, *J*=12.1 Hz, 1H)	13, 14, 17 14, 16, 35, 36
16	C	C–O	82.3	82.3		
17	CH	CH–O	81.4	81.4	3.66–3.72 (m, 3H)	
18	CH2	CH_2	25.9	25.9	1. 76–1. 83 (m, 4H), 1.98–2.04 (m, 3H)	17, 19
19	CH2	CH_2	29.5	29.5	2.18–2.22 (m, 2H), 1.42–1.46 (m, 3H)	18, 20, 34
20	C	C–O	84.7	84.8		
21	CH	CH–O	85.2	85.2	4.34 (d, *J*=3.5 Hz, 1H)	19, 20, 33
22	CH	CH	35.0	35.1	2.30–2.39 (m, 4H)	
23	CH2	CH_2	32.0	32.3	2.30–2.39 (m, 4H), 1.42–1.46 (m, 3H)	24, 33 21, 22, 33
24	CH	CH–O	74.4	76.4	4.36 (t, *J*=6.6 Hz, 1H)	25
25	CH	CH–O	77.4	76.8	3.66–3.72 (m, 3H)	24
26	CH	CH	32.3	31.8	1.30–1.38 (m, 3H)	28
27	CH2	CH_2	37.2	37.1	1.30–1.38 (m, 3H) 1.30–1.38 (m, 3H)	26
28	CH	CH	36.7	36.4	1.53 (br, 1H)	
29	C	O–C–OH	97.1	97.2		
30	CH2	CH_2–OH	67.0	66.9	3.93(d, *J*=11.9 Hz, 1H), 3.33(d, *J*=11.9 Hz, 1H)	29
31	CH3	CH_3	16.4	16.4	0.87–0.89 (m, 6H)	29
32	CH3	CH_3	17.0	17.0	0.82 (d, *J*=5.8 Hz, 3H)	25, 26, 27
33	CH3	CH_3	16.1	16.1	0.87–0.89 (m, 6H)	21, 22, 23
34	CH3	CH_3	22.7	22.8	1.15 (s, 3H)	19, 20
35	CH3	CH_3	27.7	29.0	1.58 (s, 3H)	15, 16
36	CH3	CH_3	13.3	13.4	0.92–0.93 (m, 4H)	13, 14, 15
37	CH3	CH_3	13.0	13.1	1.00–1.03 (m, 4H)	11, 12, 13
38	CH3	CH_3	11.5	11.5	0.95 (d, *J*=7.0 Hz, 3H)	3, 4
39	CH3	CH_3	14.4	14.4	0.97 (d, *J*=6.9 Hz, 3H)	1, 2, 3
40	CH3	O–CH_3	59.5	59.5	3.64 (s, 3H)	11

[a] Published by Xiao and Huang (2002)

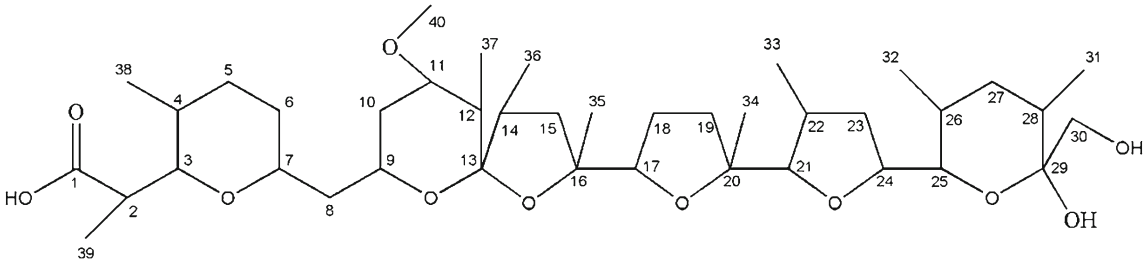

Fig. 4 Chemical structure of NIG355

2.39–2.30 (m, 4H), 2.22–2.18 (m, 2H), 2.04–1.98 (m, 3H), 1.83–1.76 (m, 4H), 1.64 (t, J=12.1 Hz, 1H), 1.58 (s, 3H), 1.53 (br, 1H), 1.46–1.42 (m, 3H), 1.38–1.30 (m, 3H), 1.15 (s, 3H), 1.09 (d, J=12 Hz, 1H), 1.04–1.00 (m, 4H), 0.97 (d, J=6.9 Hz, 3H), 0.95 (d, J=7.0 Hz, 3H), 0.93–0.92 (m, 4H), 0.89–0.87 (m, 6H), 0.82 (d, J=5.8 Hz, 3H). A preliminary structure of NIG355 can be built up progressively by ^{1}H–^{1}H COSY (correlated spectroscopy; Fig. S4), HMBC (heteronuclear multiple-bond correlation; Fig. S5) and HSQC (heteronuclear singular quantum correlation; Fig. S6) correlations. And the structure of NIG355 derived from above analysis is shown in Fig. 4.

Discussion

In predicting or mitigating HABs, it is important to understand which factors are essential in controlling the outbreak and disappearance of HABs (Blauw et al. 2006). Recently, data analysis using various models has been widely applied for the prediction of HABs, thereby providing a helpful tool for evaluating the relative importance of each factor in outbreak and disappearance processes (Glibert et al. 2010; Lane et al. 2009; McGillicuddy 2010; Raine et al. 2010). However, the mechanism behind the development and decline of an individual bloom event remains incompletely understood despite the numerous studies on the relationship between HABs and environmental factors, such as temperature, light intensity, and inorganic nutrient.

Marine bacteria are considered to be one of the key biological agents in the dramatic termination of algal blooms (Doucette 1995; Schoemann et al. 2005; Toncheva-Panova and Ivanova 2000), prompting the identification of bacteria capable of controlling HABs in coastal regions. Several strains of bacteria that could inhibit or kill HAB species had been isolated (Kim et al. 2008; Mayali and Azam 2004; Park et al. 2010; Su et al. 2007). However, few studies on actinomycetes with algicidal activity against HAB species have been reported to date. In the present study, strain O4-6, which has high algicidal activity (95.4 %) against *P. globosa*, was isolated and initially identified as *S. malaysiensis* O4-6. The algicidal activity exhibited by *S. malaysiensis* supplements its biological activity, as Cheng et al. (2010) found that *S. malaysiensis* MJM1968 showed antifungal activity, demonstrating that the species could potentially produce more active substances. The strain O4-6, as reported in the present study with wide ranges of temperature (20–40 °C), pH (5.0–10.0), and salinity (both freshwater and seawater) tolerance, should have the ability to produce different active substances under specific conditions.

The action modes of reported algicidal bacteria could be summarized as direct (bacterial and algal cell contact) and indirect (extracellular secretion of algicidal substances) interactions (Mayali and Doucette 2002). Nevertheless, regardless of action mode, active compounds would be needed against the host algae in different mechanisms. The algicidal compounds excreted by bacteria identified thus far mainly include proteins (Lee et al. 2000), peptides

Table 4 Comparison of activities of NIG355 with other reported biological algicides

Algcide	Target species	MIC (μg ml^{-1})	Source or reference
NIG355	*Phaeocystis globosa*	0.5±0.1	This study
	Alexandrium tamarense	2±0.3	This study
	Chlorella autotrophica	>10	This study
Sophorolipid	*A. tamarense*	20	Sun et al. (2004)
Ethyl 2-methylacetoacetate (EMA)	*C. pyrenoidosa*	>0.49[a]	Li and Hu (2005)
	C. vulgaris	>10[a]	Li and Hu (2005)
L-Lysine	*Microcystis aeruginosa*	6	Yamamoto et al. (1998)
β-Cyanoalanine	*Oscillatoria amphibia*	3.1	Yoshikawa et al. (2000)
	M. aeruginosa	12.5	Yoshikawa et al. (2000)

Mean values±standard deviation are given where applicable

[a]Values was in EC_{50}, rather than MIC

(Jeong et al. 2003), amino acids (Yoshikawa et al. 2000), antibiotics (Kawano et al. 1997), and pigments (Kim et al. 2008). The number of determined algicidal compounds is significantly lower than that of algicidal bacteria, which can be attributed to the difficulty of purifying active compounds from algicidal bacteria or the limited types of algicidal substances excreted by bacteria. The author tended to believe the latter reason, and we thus focused on actinomycetes, which can produce many bioactive secondary metabolites, to obtain more algicidal substances.

NIG355 produced by the high algicidal actinomycete (*S. malaysiensis* O4-6), was further purified and identified in this article. The compound has the similar NMR data of M2 (Table 3), demonstrating their highly similarity in structure. However, inspection of the slight difference of δ(C) for C-4, C-11, C-35 (Table 3) revealed that these two compounds might have different configuration. Of course, errors caused by different instruments, technician or sample purity should also be considered. In fact, M2 was determined as Nigericin by Xiao and Huang (2002) based on their NMR, IR and UV Data, despite the differences of C chemical shifts between the two compounds. However, the author supposed that NIG355, having almost the same δ(C) differences like M2 with Nigericin, might be one stereoisomer of the known compound (Nigericin). And the 18 chiral C atoms in their structure can also indicate a high potential of diversity in configuration. But, further research is still needed to illustrate the spatial configuration of NIG355. Comparisons of NIG355's algicidal activity with those of other reported biological algicides showed that NIG355 significantly inhibited the growth of *P. globosa* and *A. tamarense*, indicating its stronger algicidal activity (Table 4). On the other hand, it had much less effect on *C. autotrophica*, highlighting its species-specific effect.

With regard to its future use, the biological safety of NIG355 should be fully considered. Nigericin could be degraded in three steps (Delort et al. 1988), which means NIG355 might also be a biodegradable substance and not cause secondary pollution, unlike other chemicals. As Grabley et al. reported (1992), in relation to nigericin, the substitution of C-1 and C-30 hydroxyl groups by chlorine exhibited only one-tenth of its cytotoxicity but one-fourth of its activity against bacteria and viruses (Grabley et al. 1992). Thus, the development of specific and environment-friendly derivatives from NIG355 for use in the control of HABs is also possible and necessary.

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