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Isolation and Evaluation of Terrestrial Fungi with Algicidal Ability from Zijin Mountain, Nanjing, China

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Approximately 60 fungal isolates from Zijin Mountain (Nanjing, China) were screened to determine their algicidal ability. The results show that 8 fungi belonging to Ascomycota and 5 belonging to Basidiomycota have algicidal ability. Of these fungi, *Irpex lacteus* T2b, *Trametes hirsuta* T24, *Trametes versicolor* F21a, and *Bjerkandera adusta* T1 showed strong algicidal ability. The order of fungal chlorophyll-*a* removal efficiency was as follows: *T. versicolor* F21a > *I. lacteus* T2b > *B. adusta* T1 > *T. hirsuta* T24. In particular, *T. versicolor* F21a completely removed algal cells within 30 h, showing the strongest algicidal ability. The results also show that all 4 fungal species degraded algal cells through direct attack. In addition, most of the tested fungi from the order Polyporales of Basidiomycota exhibited strong algicidal activity, suggesting that most fungi that belong to this order have algicidal ability. The findings of this work could direct the search for terrestrial fungi for bloom control.

***Keywords*:** algae, algal control agents, algicidal ability, fungi

Harmful algal blooms seriously damage fisheries, reduce species diversity, affect drinking water supply and public health, and cause losses in recreational amenities, among others. Three main approaches to control algal blooms in freshwater environments are currently available to address their adverse effects: nutrient limitation, direct eradication, and biomanipulation (Sigee, 2005). Nutrient limitation should be the main goal of any management plan, but it takes years to achieve results, thus restricting the ability of this method to immediately control bloom events (Boston and Hill, 1991). Direct eradication is a short-term solution, but it requires mechanical methods to remove algae directly from the aquatic environment or chemicals to inhibit/destroy algal cells. In addition, their high-energy inputs, inevitable damage to the aquatic environment, and detrimental side effects on aquatic organisms (Jeong *et al.*, 2000; Mason, 2002) render direct methods undesirable.

Biomanipulation is recognized as a short-term but effective ecological strategy of eliminating or reducing the growth of harmful algae with the addition of algal antagonistic organisms (biological control agents) (Sigee, 2005). Different types of biological control agents, such as viruses, fungi, and bacteria, have been investigated for their ability to inhibit algal blooms. To date, most biological studies have mainly concentrated on screening bacteria and cyanophages with the ability to control blooms in aquatic environments (Ahn *et al.*, 2003; Mayali and Azam, 2004; Kang *et al.*, 2005; Mu *et al.*, 2007; Dillon and Parry, 2008).

The method of controlling blooms with bacteria or cyanophages also has inherent liabilities, and its application has remained limited. Previous research has attempted to isolate new strains of bacteria or cyanophages, whereas the search for fungi with algicidal ability on cyanobacteria has not been given much attention (Jia *et al.*, 2010; Wang *et al.*, 2010b). Some studies have shown that some fungi are algal parasites, which can be an important factor in controlling seasonal phytoplankton succession (Van Donk, 1989), whereas others have reported that fungi can produce antibiotics to lyse cyanobacteria (Redhead and Wright, 1978, 1980). Recently, Jia *et al.* (2010) reported on *Trichaptum abietinum* 1302BG, a strain of white-rot fungus that could directly degrade algal species, and Wang *et al.* (2010b) identified *Lopharia spadicea* as capable of damaging algal species. These two fungi belong to the order Polyporales of the phylum Basidiomycota. Whether other fungi have the same algae-degrading ability and whether such fungi have better or worse degradation efficiency than currently known species are interesting research topics. In the present study, approximately 60 fungal isolates from Zijin Mountain (Nanjing, China) were screened to determine their algicidal ability.

Materials and Methods

Algal cultures

A unialgal culture of *Microcystis aeruginosa* PCC7806 was provided by the Freshwater Algae Culture Collection of the Chinese Academy of Sciences. The strain was maintained in an illumination incubator for 7 d at 25°C and under a 12 h:12 h light/dark cycle with approximately 90 mol photons/m^2/sec provided by cool white fluorescent lamps to achieve exponential growth. A BG-11 growth medium was used: 150 mg $NaNO_3$, 4 mg K_2HPO_4, 7.5 mg $MgSO_4 \cdot 7H_2O$, 3.6 mg $CaCl_2 \cdot 2H_2O$, 5.8 mg $Na_2SiO_3 \cdot 9H_2O$, 0.6 mg citric acid, 0.6 mg ferric ammonium citrate, 0.1 mg EDTA, 2 mg Na_2CO_3, 1 ml A_5 solution

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Table 1. Preliminary test results on chlorophyll-*a* content changes in the algal cultures after 120 h of co-incubation

Phylum/ subphylum	Class	Order	Family	Species	Accession number	Closest relatives in NCBI	ITS identity (%)	Chlorophyll-*a* removal efficiency (%)
Ascomycota	Dothideomycetes	Botryosphaeriales	Byophaeriaceae	*Botryosphaeriaceae* sp. F16	JF439465	*Botryosphaeria obtusa* strain CBS119049	99.5	81.04±1.03
				Botryosphaeriaceae sp. F30	JF439466	*Botryosphaeria obtusa* strain CBS119049	99.5	49.60±0.98
		Capnodiales	Davidiellaceae	*Cladosporium cladosporioides* F08	JF439469	*Cladosporium cladosporioides*	99.7	0
		Dothideales	Dothioraceae	*Aureobasidium pullulans* F11	JF439462	*Aureobasidium pullulans*	98.9	0
				A. pullulans F42	JF439463	*Aureobasidium* sp. TMS-2011 voucher SC8d50p14-8	99.4	0
		Pleosporales	Montagnulaceae	*Paraconiothyrium brasiliense* F01	JF439492	*Paraconiothyrium brasiliense* strain STE-U 6306	99.8	8.98±0.12
	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus aculeatus* F36	JF439460	*Aspergillus aculeatus* isolate UOA/HCPF 9163A	99.8	73.89±2.23
				Aspergillus niger F34	JF439461	*Aspergillus niger* contig An03c0110	100	0
				Penicillium janthinellum F48	JF439495	*Penicillium janthinellum* isolate GFI 47	99.8	82.25±3.12
				Penicillium sp. F02	JF439496	*Penicillium sumatrense* strain UWFP 700	100	0
				Penicillium sp. F03	JF439497	*Penicillium multicolor* isolate M2	100	0
				Penicillium sp. F04	JF439498	*Penicillium sclerotiorum* strain NRRL 32583	97.7	0
				Penicillium sp. F06	JF439493	*Penicillium* sp. I1Z	100	85.43±4.34
				Penicillium sp. F07	JF439494	*Penicillium canescens* strain NRRL 910	99.1	76.49±3.23
				Penicillium sp. F27	JF439499	*Penicillium daleae* strain NRRL 922	98.3	35.07±1.34
				Penicillium sp. F33	JF439500	*Penicillium* sp. HZ-5	95.4	21.82±0.88
				Penicillium sp. F35	JF439501	*Penicillium* sp. GZU-BCECYN62-5	97.6	21.80±0.97
				Penicillium sp. F40	JF439502	*Penicillium* sp. GZU-BCECYN62-5	97.0	49.67±0.75
				Penicillium virgatum F22	JF439503	*Penicillium virgatum* strain IHB F 536	100	82.82±1.89
	Leotiomycetes	Helotiales	Myxotrichaceae	*Geomyces* sp. F12	JF439475	*Geomyces destructans* isolate MmyotCH-4	97.7	0
				Geomyces sp. F29	JF439476	*Geomyces* sp. T489/9b	100	5.37±0.11
				Leotiomycetes sp. F21	JF439482	*Potebniamyces pyri* strain CBS 473.81	96.9	25.60±0.12
	Sordariomycetes	Hypocreales	Nectriaceae	*Fusarium oxysporum* F19	JF439472	*Fusarium oxysporum* isolate d20	100	38.16±0.45
				F. oxysporum F23	JF439473	*Fusarium oxysporum* strain BD	99.7	64.64±0.98
				F. oxysporum F49	JF439474	*Fusarium oxysporum* strain BD	99.7	0
				Hypocreales sp. F18	JF439480	*Neonectria ramulariae* strain CBS 730.87	85.8	28.88±0.77
			Hypocreaceae	*Hypocrea koningii* F50	JF439478	*Hypocrea koningii* strain ATCC 64262	99.5	26.23±0.33
				Hypocrea sp. F31	JF439479	*Hypocrea virens* strain GL-9	92.1	0
				Trichoderma atroviride F39	JF439513	*Trichoderma atroviride* isolate MIAE00220	99.5	0
				Trichoderma spirale F24	JF439514	*Trichoderma spirale* strain DAOM 183974	99.1	14.28±0.12
				T. spirale F28	JF439515	*Trichoderma spirale* strain DAOM 183974	99.7	33.19±0.54
				Trichoderma virens F41	JF439516	*Hypocrea virens* strain GL-13	99.7	20.76±0.11
		Sordariales	Chaetomiaceae	*Chaetomium nigricolor* F25	JF439467	*Chaetomium nigricolor* strain Dzf15	99.8	0
				Chaetomium sp. F26	JF439468	*Chaetomium* sp. B132	96.5	5.94±0.56
		Xylariales	Amphisphaeriaceae	*Pestalosphaeria hansenii* T11	JF439505	*Pestalosphaeria hansenii*	100	10.57±0.67
				Pestalotiopsis sp. F20	JF439506	*Pestalotiopsis microspora* isolate WT98	99.0	0
				Pestalotiopsis sp. F32	JF439507	*Pestalotiopsis vismiae* isolate XSD-31	99.5	89.45±2.34
				Pestalotiopsis sp. F44	JF439508	*Pestalotiopsis vismiae* isolate XSD-31	99.4	0
Basidiomycota	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophyllum commune* T28	JF439509	*Schizophyllum commune* isolate Z3	99.9	16.49±0.89
				S. commune T31	JF439510	*Schizophyllum commune* isolate Z3	100	58.57±0.11
		Polyporales	Steccherinaceae	*Irpex lacteus* T2b	JF439481	*Irpex lacteus* isolate T60	99.7	99.1±0.95
			Coriolaceae	*Fomitopsis pinicola* T25	JF439471	*Fomitopsis pinicola* strain xsd08136	99.6	88.05±2.45
				Trametes hirsuta T24	JF439511	*Trametes hirsuta* strain lg-9	99.5	99.2±0.66
				Trametes versicolor F21a	JF439512	*Trametes versicolor* strain BCRC 36089	99.8	100±0.00
		Russulales	Meruliaceae	*Bjerkandera adusta* T1	JF439464	*Bjerkandera adusta* strain dd08030	99.4	98.27±3.23
			Peniophoraceae	*Peniophora incarnata* F35-1	JF439504	*Peniophora incarnata* isolate F20	96.9	15.06±1.21
Mucoromycotina		Mucorales	Cunninghamellaceae	*Cunninghamella elegans* T4	JF439470	*Cunninghamella elegans* strain MUCL 16087	99.3	0
			Mucoraceae	*Gongronella* sp. F39	JF439477	*Gongronella butleri* strain F13	94.6	0
		Mortierellales	Mortierellaceae	*Mortierella alpina* T2	JF439483	*Mortierella alpina* strain CBS 224.37	99.2	4.28±0.09
				M. alpina T7	JF439484	*Mortierella alpina* strain CBS 224.37	98.9	0
				Mortierella humilis F13	JF439486	*Mortierellales* sp. WD7C	100	0
				Mortierella elongata F15	JF439485	*Mortierella elongata* strain NBRC 8570	99.7	0
				Mortierella sp. T3	JF439488	*Mortierella alpina* strain CBS 224.37	99.3	19.94±0.23
				Mortierella sp. T6	JF439489	*Mortierella* sp. Ppf23	99.4	0
				Mortierella sp. T8	JF439490	*Mortierella alpina* strain CBS 224.37	99.0	0
				Mortierella sp. T10	JF439491	*Mortierella alpina* strain CBS 224.37	99.3	0
				Mortierella sp. T12	JF439487	*Mortierella alpina* strain CBS 224.37	99.3	0
Unknown fungus				F14	JF439517	*Entomocorticium* sp. G isolate TSpGB1067	84.4	0

Accession number, the accession number of the isolated fungi.

(286 mg H_3BO_3, 181 mg $MnCl_2{\cdot}4H_2O$, 22 mg $ZnSO_4{\cdot}7H_2O$, 7.9 mg $CuSO_4{\cdot}5H_2O$, 3.9 mg $Na_2MoO_4{\cdot}2H_2O$, and 100 ml distilled water) with Co, and 99.9 ml distilled water, pH 7.0 (Rippka *et al.*, 1979).

Fungal isolation, identification, and maintenance

The top soils and litter (0-20 cm) of the forest floor of Zijin Mountain (Nanjing, China) were collected (Song *et al.*, 2004). The dilution plate method was used to isolate fungal strains on Rose Bengal Agar plates. Each colony was maintained on petri dishes with potato dextrose agar (PDA) medium (4 g/L potato starch, 20 g/L dextrose, and 15 g/L agar). Each isolate was identified by morphological traits and molecular methods. The actively growing mycelium of each representative isolate in PDA plates was used for DNA extraction with the modified CTAB method (Kuhad *et al*., 2004). The ITS regions (partial sequence of 18S rRNA, ITS1, 5.8S rRNA, ITS2, and partial sequence of 28S rRNA) were amplified using the primer pair ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′)/ITS4 (5′-TCCTCCGCTT ATTGATATGC-3′) (White *et al*., 1990; Gardes and Bruns, 1993). The reaction mixtures (50 μl) contained 1 μl of 50-200 ng fungal genomic DNA, 2 μl of 10 mmol/L primer, 5 μl 10× PCR buffer (including Mg^{2+}), 1 μl of 10 mmol/L dNTPs, 0.4 μl Taq DNA polymerase (5 U/μl, Taq Plus, Sangon), and 38.6 μl of ddH_2O. The thermal cycling conditions included initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, and primer extension at 72°C for 1 min, followed by one final cycle of primer extension at 72°C for 5 min. The PCR products were separated on 1% agarose gel by electrophoresis. The suitable amplified band was purified using a DNA Gel Extraction kit (QIAquick Gel Extraction Kit, Germany) and then inserted into a pMD19-T vector (TaKaRa) according to the manufacturer's instructions. After the recombinant vectors were transformed into the *Escherichia coli* DH5α strain, the clone containing the inserted fragment was detected by PCR with the ITS1F/ITS4 primer pair and the positive clone was analyzed using an ABI 3730 capillary sequencer (Applied Biosystems, USA) with M13(+)/(-) universal primers. The nucleotide sequences of the isolates were analyzed with the BlastN search program from the National Center for Biotechnology Information (NCBI) website.

A sample of round fungal mycelium from a PDA solid plate cut using a 7 mm hole puncher was inoculated into a 9 cm plate containing 15 ml of potato liquid medium and cultivated under static conditions. After 5 d, the mycelial films in the plates were used as inocula for algicidal experiments.

Evaluation of algicidal ability

Batch liquid tests were conducted in 250 ml Erlenmeyer flasks containing 100 ml of algal medium and a fungal inoculum. Experiments were carried out with *M. aeruginosa* PCC7806 (cyanobacterium) to evaluate the algicidal effects of each fungus. The initial chlorophyll-*a* concentration used for screening was approximately 600-700 μg/L. The algicidal experiments were conducted at 25°C, 90 μmol photons/m/sec, and 120 rpm. A system without fungi served as the control. Total chlorophyll-*a* was extracted with 90% acetone and determined according to the Standard Methods for the Examination of Water and Wastewater (1998). All experiments were conducted in triplicate.

Determination of algicidal mode

Different specimens were inoculated into a 100 ml algal medium to compare algicidal efficiencies and determine the algicidal mode of strong algicidal fungi. The added specimens were living fungal films, autoclaved fungal films, and 5 ml of 0.22 μm Millipore membrane fungal culture filtrates.

Spectrophotometric analysis

Changes in the absorption spectra (300-1,000 nm) of the cultures were monitored using a DU 800 spectrophotometer to analyze the degradation products of the medium after 60 h of co-incubation.

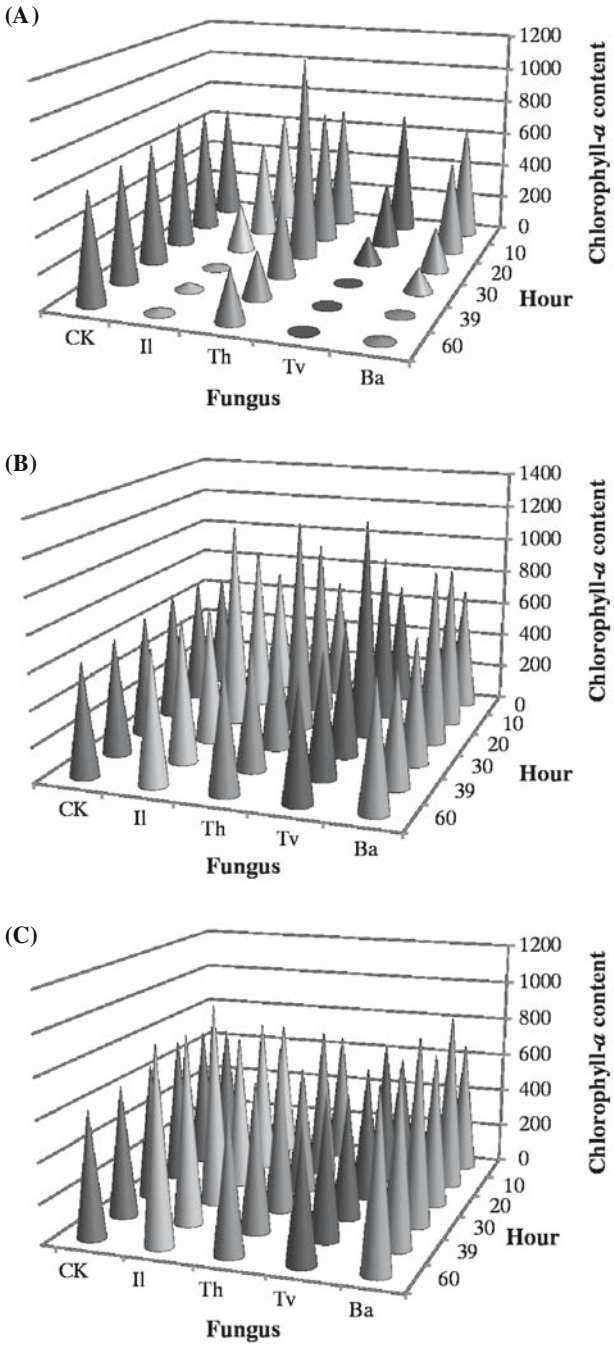

Fig. 1. Impact of fungal strain on algal cultures: (A) living fungal films; (B) dead fungal films; (C) fungal culture filtrates. CK, algal culture without fungal films; Ba, *B. adusta* T1; Il, *I. lacteus* T2b; Th, *T. hirsuta* T24; Tv, *T. versicolor* F21a. Chlorophyll-*a* content (μg/L)

Table 2. Chlorophyll-*a* content of the co-culture with living mycelia (Mean±SD)

Fungus	Chlorophyll-*a* of the co-culture (μg/L)					
	0 h	10 h	20 h	30 h	39 h	60 h
CK	655.45±31.31	710.73±8.53	729.76±31.60	679.84±12.10	652.44±24.60	621.40±20.97
I. lacteus T2b	646.25±19.11	548.61±19.98	272.97±8.44	20.53±1.27	39.00±1.69	24.17±1.17
T. hirsuta T24	705.19±15.45	763.55±16.89	1154.35±35.91	472.37±24.74	260.07±14.27	280.73±14.19
T. versicolor F21a	701.33±13.50	366.77±11.57	153.55±3.50	0.07±0.01	0.67±0.02	0.00±0.00
B. adusta T1	656.28±26.78	520.12±17.67	243.85±7.05	132.81±67.04	10.85±5.68	17.72±1.44

CK, algal culture without fungus.

Fungal biomass variation

The biomass variation test was conducted by oven-drying mycelial films to a weight before and after 60 h of cultivation in algal cultures.

Statistical analysis

Significant differences in cell density and chlorophyll-*a* content between the treated strains and the control were analyzed using one-way ANOVA with SPSS 17.0, where $p<0.01$ was considered statistically significant. All data were expressed as the Mean±SD of triplicate experiments.

Results

Isolation and identification of terrestrial fungi

The isolated fungi were first identified according to their morphological traits and then validated by the sequence information on the ITS regions. In total, 58 fungal species were isolated (Table 1). Among these, 38 belong to the phylum Ascomycota, 8 belong to the phylum Basidiomycota, and 11 belong to the subphylum Mucoromycotina; 1 fungus was not classified. Most species shared sequence similarities ≥98% (data are available from the NCBI). The fungus *Hypocreales* sp. F18 and the unknown fungus F14 shared very low sequence similarities with their closest relatives in the NCBI database (85.8% and 84.4%, respectively). The two fungi are either novel species or members of some new genera.

Preliminary screening of algicidal fungi

The above-mentioned fungal isolates (~60) were tested in three batches for their algicidal ability. As shown in Table 1, 13 of the isolates removed more than 60% of the algal chlorophyll-*a* content after a 120 h co-incubation. These isolates include 8 fungi (*Botryosphaeriaceae* sp. F16, *Aspergillus aculeatus* F36, *Penicillium janthinellum* F48, *Penicillium* sp. F06, *Penicillium* sp. F07, *Penicillium virgatum* F22, *Fusarium oxysporum* F23, and *Pestalotiopsis* sp. F32) belonging to Ascomycota and 5 fungi (*Irpex lacteus* T2b, *Fomitopsis pinicola* T25, *Trametes hirsuta* T24, *Trametes versicolor* F21a, and *Bjerkandera adusta* T1) belonging to Basidiomycota. Of these fungi, *I. lacteus* T2b, *T. hirsuta* T24, *T. versicolor* F21a, and *B. adusta* T1 removed more than 95% of the algal cells after 120 h of co-incubation (strong algicidal fungi). The fungal isolates belonging to the subphylum Mucoromycotina did not show significant algicidal activity. Interestingly, all the strong algicidal fungi belong to Basidiomycota, and most of the tested fungi belonging to the order Polyporales of this phylum showed strong algicidal activity.

Algicidal ability of strong algicidal fungi

The four newly isolated fungi that showed strong activity were investigated under the same conditions/batches for comparison. As shown in Fig. 1A and Table 2, the initial chlorophyll-*a* content of the algal cells ranged between 646.25 and 706.85

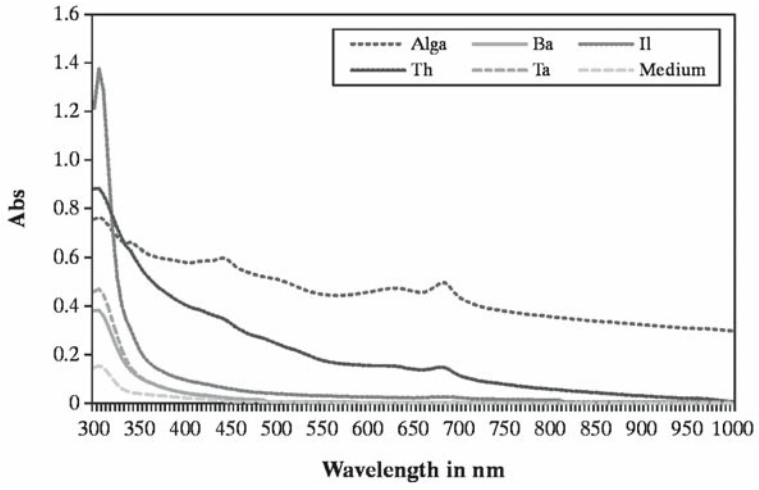

Fig. 2. Absorbance spectra of the algal culture after 60 h of fungal treatment. Alga, algal culture without fungal films; Ba, *B. adusta* T1; Il, *I. lacteus* T2b; Th, *T. hirsuta* T24; Tv, *T. versicolor* F21a; Medium, the BG-11 medium.

Table 3. Weight changes in the fungal mycelial films

Fungus	Initial weight (g)	End weight (g)
I. lacteus T2b	0.1418±0.0057	0.1574±0.0255
T. hirsuta T24	0.0875±0.0088	0.0886±0.0049
T. versicolor F21a	0.0976±0.0049	0.1091±0.0129
B.adusta T1	0.0784±0.0027	0.0889±0.0077

End weight, weight of the fungal film after co-incubation with algal cells for 60 h. The end weights of all films were not significantly different from that of the initial weights ($p>0.05$).

μg/L. After 60 h of incubation, only the medium treated with *T. versicolor* F21a was reduced to 0 μg/L within 60 h. *T. versicolor* F21a removed almost all the algal cells within 30 h, whereas *I. lacteus* T2b removed 96% of the algal cells within the same period. *B. adusta* T1 removed almost all the algal cells within 39 h, but with *T. hirsuta* T24, approximately 39% chlorophyll-*a* (280 μg/L) remained within 60 h. All four living fungal mycelial films tested significantly inhibited algal growth within 60 h ($p<0.01$). The fungal chlorophyll-*a* removal efficiency was thus of the order *T. versicolor* F21a > *I. lacteus* T2b > *B. adusta* T1 > *T. hirsuta* T24.

Determination of algicidal mode of strong algicidal fungi

The addition of dead films slightly increased the chlorophyll-*a* content in the first 20 h of inoculation and then reduced it to almost the initial contents (Fig. 1B). The addition of fungal cultural filtrates of the four fungi did not inhibit and slightly increased the growth of *M. aeruginosa* (Fig. 1C). These results indicate that the four fungi acted through direct attack.

Spectrophotometric analysis of the degradation products

The spectra of co-cultural degradation products driven by 4 ungi after 60 h of cultivation were obtained at wavelengths ranging from 300 to 1,000 nm. Figure 2 shows that the major visible light absorbance peaks (OD_{680}) of the algal cells completely disappeared after 60 h of treatment with *T. versicolor* F21a, *I. lacteus* T2b, and *B. adusta* T1. The spectra of the fungus-treated cultures were similar to those of the BG-11 culture in the visible region (400-1,000 nm) and were larger than those of the BG-11 medium in the invisible region (<400 nm). The results suggest that most algal cells treated with these fungi were finally degraded into small molecules. The major visible light absorbance peaks (OD_{680}) of the algal cells that were treated with *T. hirsuta* T24 did not completely disappear, suggesting that the fungus partially degraded the algal cells within 60 h.

Variations in biomass of strong algicidal fungi during incubation

After preculturing for 5 d in 9 mm plates, the dry weights of the mycelial films from *B. adusta* T1, *T. hirsuta* T24, and *T. versicolor* F21a were determined to be between 0.0784 and 0.0976 g (Table 3). The dry weight of the films for *I. lacteus* T2b was 0.1418 g. The biomasses of the four fungi slightly increased (by ~10%), but the changes were not significantly different from their initial weights ($p>0.05$).

Discussion

In the present work, 13 fungal isolates (~20% of all isolates) exhibited algicidal ability. Eight of these fungi belong to Ascomycota; the remaining species belong to Basidiomycota, of which *I. lacteus* T2b, *T. hirsuta* T24, *T. versicolor* F21a, and *B. adusta* T1 are the four strongest algicidal fungi among the isolates studied. In particular, most of the strong algicidal fungi and the algicidal fungi *T. abietinum* 1302BG and *L. spadicea* belong to the order Polyporales, suggesting that most fungi in this order have algicidal ability. Species within Polyporales are saprotrophic fungi, which are mostly wood rotters. Many fungi in this order had been investigated for the control or remediation of environmental hazardous materials in soils or water bodies, e.g., polycyclic aromatic hydrocarbons, textile dye effluents, and endocrine-disrupting compounds (Abadulla *et al.*, 2000; Zjalic *et al.*, 2006; Couto, 2007; Cajthaml *et al.*, 2009; Haritash and Kaushik, 2009; Novotny *et al.*, 2009; Anastasi *et al.*, 2010). The observation that most of the strong algicidal fungi belong to Polyporales is in accordance with the strong decomposition of saprotrophic fungi. Many saprotrophic fungi show strong degradation ability toward harmful algae.

Previous research has reported that some bacteria and fungi inhibit the growth of algae or degrade algae. Ahn *et al.* (2003) reported that a *Bacillus subtilis* C1 culture containing 10 mg/L surfactin completely inhibited the growth of *M. aeruginosa* within 48 h. Mu *et al.* (2007) reported that *Bacillus fusiformis* removed 70% of the chlorophyll-*a* content of cultures with less than 550 μg/L chlorophyll-*a* within 7 d. Jia *et al.* (2010) tested four algae and reported that *T. abietinum* 1302BG removed most of them within 48 h, whereas Wang *et al.* (2010b) reported that *L. spadicea* lysed three algal strains within 39 h. Compared with previous results, the findings of the present study indicate that *T. versicolor* F21a showed the strongest algicidal ability after eliminating almost all algal cells within 30 h.

Algicidal bacteria kill algal cells either directly or indirectly (Mayali and Azam, 2004; Kim *et al.*, 2009; Wang *et al.*, 2010a). Algal-lysing fungi have been isolated from algal habitats as early as in 1978, and the tested fungi typically indirectly inhibited algae or lysed algal cells (Redhead and Wright, 1978, 1980; Bott and Rogenmuser, 1980; Raghukumar, 1986; Nakagiri and Ito, 1997; Jenkins *et al.*, 1998). Recently, direct lysis, a new mechanism of fungus-alga interaction, was observed. Algal cells were gradually surrounded by a mucous membrane secreted by the fungal mycelial film and directly degraded by *T. abietinum* 1302BG (Jia *et al.*, 2010). In the present study, the addition of dead films or fungal cultural filtrates did not inhibit the growth of *M. aeruginosa*, indicating that the four strong fungi degraded the algal cells through direct attack. The biomasses of the four fungi slightly increased, whereas most algal cells disappeared after 60 h of co-incubation, suggesting that these fungi decomposed and utilized the algal cells for their growth. The results also indicate that *I. lacteus* T2b, *T. hirsuta* T24, *T. versicolor* F21a, and *B. adusta* T1 have an algicidal mode similar to that of *T. abietinum* 1302BG.

Conclusions

In the present study, approximately 60 fungal isolates from Zijin Mountain (Nanjing, China) were screened to determine their algicidal activity. Eight fungi belonging to Ascomycota

and 5 belonging to Basidiomycota were found to have algicidal ability. Most of the strong algicidal fungi belong to the order Polyporales of the phylum Basidiomycota. Of these fungi, *I. lacteus* T2b, *T. hirsuta* T24, *T. versicolor* F21a, and *B. adusta* T1 showed strong algicidal ability. The fungus *T. versicolor* F21a showed the best potential for controlling cyanobacterial blooms as it completely eliminated algal cells within 30 h. The results of this work add more biological control agents to current knowledge and direct the search for terrestrial fungi for algal control.

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