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Algicidal efficiency and mechanism of *Phanerochaete chrysosporium* against harmful algal bloom species

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ABSTRACT

Algal blooms pose serious ecological problems, threaten biological safety, and cause great economic losses. While white-rot fungi have attracted worldwide attention for their ability to inhibit algal blooms, the algicidal efficiency and mechanism of *Phanerochaete chrysosporium* have not been reported to date. In this study, *Cryptomonas obovata* FACHB-1301, *Oscillatoria* sp. FACHB-1083, and *Scenedesmus quadricauda* FACHB-507 were co-cultured with *P. chrysosporium* under optimum conditions with a fungal dosage of 250 mg^{-1}, pH of 7.0 and dissolved oxygen content of 7.0 mg^{-1}. The results showed that the chlorophyll-a content, dehydrogenase activity, and soluble protein content of the three algae decreased, whereas the malondialdehyde content increased after 48 h of co-culture. Flow cytometry showed that *P. chrysosporium* damaged the membranes and nucleic acid of the algal cells, thereby causing cell death. Fourier transform infrared spectroscopy results indicated that the pyrrole ring of chlorophyll-a was degraded, and scanning electron microscopy revealed that the structure of the algal cells was seriously damaged by P. chrysosporium. These results demonstrated that the algal cells were severely damaged by *P. chrysosporium* and were finally degraded directly by the fungus, which has superior properties as algicidal agent for its low cost and safety compared to physical and chemical methods.

1. Introduction

Algal blooms, which are characterized by abnormal reproduction of algae in aquatic ecosystems and other aquatic organisms, are one of the most serious environmental problems observed in lakes, reservoirs, and other natural bodies of water [1,2]. They deteriorate water quality, block the physiological processes of aquatic organisms, damage the structure of the aquatic ecosystem, and impair the functions of the body of water and the ecological environment. Moreover, algal blooms can seriously affect the utilization of water resources by industry, agriculture, aquaculture, and water transportation, and they pose a threat to animals, plants, and humans [3,4].

Many studies have explored physical and chemical methods of inhibiting algal blooms. However, physical methods cannot completely eliminate the blooms and are expensive, while chemical methods are prone to secondary pollution and poor security. Thus, biological methods, which are inexpensive and safer, are considered a promising approach to control algal blooms [5]. White-rot fungi can degrade and break down various environmental pollutants, including dyes, lignin, and toxic wastes [6,7]. Previous studies have indicated that these fungi can also have a suppressive effect on algal species due to fungal secretions [8,9]. To date, the algicidal efficiency and mechanism of *Phanerochaete chrysosporium*, a fungus with algal bloom-inhibiting properties, have not been reported.

The aims of the present study were to investigate the algicidal efficiency of the white-rot fungus *P. chrysosporium*, as well as the mechanism by which it inhibits algal blooms. Structural characteristics, including the algal cell and surface morphology, were also assessed and compared via flow cytometry (FCM), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

2. Materials and methods

2.1. Algal strains and culture conditions

Cryptomonas obovata FACHB-1301, *Oscillatoria* sp. FACHB-1083, and *Scenedesmus quadricauda* FACHB-507, which are dominant species in the Fu Niu Lake in China, were provided by the Freshwater Algae Culture Collection of the Chinese Academy of Sciences. All strains were maintained in an illumination incubator for 7 d at 25 °C on a 12 h light/12 h dark cycle with approximately 90 μmol m^{-2} s^{-1} of photons provided by cool-white fluorescent lamps to achieve exponential growth. The growth media used for *C. obovata* FACHB-1301, *Oscillatoria* sp. FACHB-1083, and *S. quadricauda* FACHB-507 are described in Tables 1, 2, and 3, respectively.

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Table 1
The growth medium used for *Cryptomonas obovata* FACHB-1301 was AF-6 (pH 6.6).

Chemical	Content (g L^{-1})	Trace metal solution	
		Medicine	Content (g L^{-1})
$NaNO_3$	0.14	$MnCl_2 \cdot 4H_2O$	0.041
KH_2PO_4	0.01	Na_2EDTA	0.75
K_2HPO_4	0.005	$FeCl_3 \cdot 6H_2O$	0.097
NH_4NO_3	0.022	$ZnCl_2 \cdot 7H_2O$	0.005
$CaCl_2 \cdot 2H_2O$	0.01	$Na_2MoO_4 \cdot 2H_2O$	0.004
Fe-citrate	0.002	$CoCl_2 \cdot 6H_2O$	0.002
$MgSO_4 \cdot 7H_2O$	0.03		
Citric acid	0.002		
Biotin	0.000002		
Thiamine HCl	0.00001		
Vitamin B_6	0.000001		
Vitamin B_{12}	0.000001		
MES	0.4		
Trace metal solution	6 mL L^{-1}		

Table 2
The growth medium used for *Oscillatoria* sp. FACHB-1083 was BG-11 (pH 7.5).

Chemical	Content (g L^{-1})	A5 solution (0.1 mL)	
		Medicine	Content (g L^{-1})
$NaNO_3$	0.15	H_3BO_3	0.286
$Na_2SiO_3 \cdot 9H_2O$	0.0058	$MnCl_2 \cdot 4H_2O$	0.181
K_2HPO_4	0.004	$ZnSO_4 \cdot 7H_2O$	0.0022
Citric acid	0.006	$CuSO_4 \cdot 5H_2O$	0.0079
EDTA	0.001	$Na_2MoO_4 \cdot 2H_2O$	0.0039
Na_2CO_3	0.002	$CoCl_2 \cdot 6H_2O$	0.002
Ferric ammonium citrate	0.0006		
$CaCl_2 \cdot 2H_2O$	0.0036		
$MgSO_4 \cdot 7H_2O$	0.0075		
A5 solution	0.1 mL L^{-1}		

Table 3
The growth medium used for *Scenedesmus quadricauda* FACHB-507 was SE (pH 7.5).

Chemical	Content (g L^{-1})	Soil extract solution	
		Medicine	Content (g L^{-1})
$NaNO_3$	0.025	H_3BO_3	0.286
NaCl	0.0025	$MnCl_2 \cdot 4H_2O$	0.181
$K_2HPO_4 \cdot 3H_2O$	0.0075	$ZnSO_4 \cdot 7H_2O$	0.0022
$MgSO_4 \cdot 7H_2O$	0.0075	$CuSO_4 \cdot 5H_2O$	0.0079
$CaCl_2 \cdot 2H_2O$	0.0025	$Na_2MoO_4 \cdot 2H_2O$	0.0039
KH_2PO_4	0.0175	$CoCl_2 \cdot 6H_2O$	0.002
$Fecl_3 \cdot 6H_2O$	0.0005		
EDTA-Na	0.0002		
Soil extract solution	40 mL L^{-1}		

2.2. Fungal strain and maintenance

P. chrysosporium was provided by the Institute of Microbiology (China). The strain was maintained on potato dextrose agar (PDA) plates for 5 d, stored at 4 °C, and then subcultured every month. After 5 d, the mycelial mats in one plate were used as an inoculum for subsequent algicidal experiments.

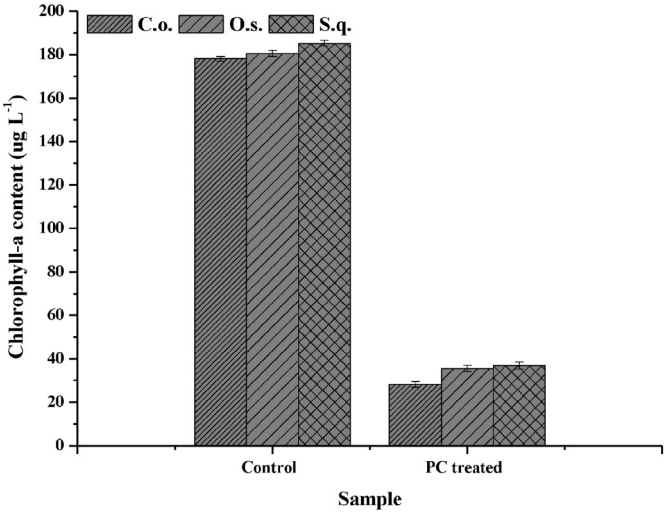

Fig. 1. Algicidal efficiency of *Phanerochaete chrysosporium* against *Cryptomonas obovata* FACHB-1301 (C.o.), *Oscillatoria* sp. FACHB-1083 (O.s.), and *Scenedesmus quadricauda* FACHB-507 (S.q.) algal species. Data are expressed as the mean ± SD (n = 3).

2.3. Inhibition tests against different algal species

Batch liquid tests were conducted in a 5000 mL beaker containing a specific dry weight of *P. chrysosporium* (250 mg L^{-1}). The same cultures as the test groups but without the fungus were used as controls throughout the experiments. The inhibition tests were conducted under conditions of 25 °C, pH 7.0, 7.0 mg L^{-1} dissolved oxygen, and 12 h light/12 h dark cycle. All experiments were conducted in triplicate.

2.4. Analytical methods

2.4.1. Biomass variation

Fifty milliliter aliquots of the samples were filtered through polycarbonate filters (diameter, 25 mm; mesh, 0.45 μm) for 48 h and then oven dried at 80 °C. The weight of each dried mycelial pellicle sample was measured. Cultures without *P. chrysosporium* served as controls before and after the algicidal experiment.

2.4.2. Total chlorophyll-a content

Total chlorophyll-a was extracted with 90% acetone and quantified using the repeated freezing–thawing extraction method [10].

2.4.3. Antioxidant enzyme assays

Twenty-five milliliter aliquots of the samples were collected and centrifuged at 8000 rpm for enzyme activity tests. The dehydrogenase activity (DHA) and soluble protein content in the supernatant were determined based on triphenyl tetrazolium chloride-dehydrogenase

Table 4
Biomass of *Phanerochaete chrysosporium* co-cultured with three algae in AF-6, BG11 and SE media (g per mycelial pellicle).

Time (h)	No inoculum controls			Treatment		
	AF-6	BG-11	SE	C.o.	O.s.	S.q.
0	0.056 ± 0.005[a]	0.067 ± 0.008[a]	0.072 ± 0.008[a]	0.069 ± 0.005[a]	0.056 ± 0.005[a]	0.056 ± 0.005[a]
48	0.064 ± 0.007[a]	0.075 ± 0.006[a]	0.084 ± 0.005[a]	0.254 ± 0.053[b]	0.182 ± 0.008[b]	0.527 ± 0.067[b]

The data indicates means ± SD (n = 3).
Cryptomonas obovata FACHB-1301 (C.o.), *Oscillatoria* sp. FACHB-1083 (O.s.) and *Scenedesmus quadricauda* FACHB-507 (S.q.) algal species.
[a] Denotes no significant difference.
[b] Denotes a significant difference with $P < 0.001$.