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Potential of extracellular enzymes from *Trametes versicolor* F21a in *Microcystis* spp. degradation

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ABSTRACT

Studies have shown that microorganisms may be used to eliminate cyanobacteria in aquatic environments. The present study showed that the white-rot fungus *Trametes versicolor* F21a could degrade *Microcystis aeruginosa*. After *T. versicolor* F21a and *Microcystis* spp. were co-incubated for 60 h, >96% of *Microcystis* spp. cells were degraded by *T. versicolor* F21a. The activities of extracellular enzymes showed that cellulase, β-glucosidase, protease, and laccase were vital to *Microcystis* spp. degradation in the early stage (0 h to 24 h), while β-glucosidase, protease, laccase, and manganese peroxidase in the late stage (24 h to 60 h). The positive and significant correlation of the degradation rate with these enzyme activities indicated that these enzymes were involved in the degradation rate of *Microcystis* spp. cells at different phases. It suggested that the extracellular enzymes released by *T. versicolor* F21a might be vital to *Microcystis* spp. degradation. The results of this study may be used to develop alternative microbial control agents for cyanobacterial control.

1. Introduction

Several cyanobacterial species are toxic and produce a diverse range of toxin [1,2], causing numerous illnesses and deaths of dogs, cattle, birds, and humans [3,4]. The increased input of nutrients into surface waters (i.e., anthropogenic eutrophication) is considered to be the main factor responsible for massive proliferations of cyanobacteria in freshwater, brackish and coastal marine ecosystems [5]. Cyanobacterial harmful algal blooms (cyanoHABs) occur worldwide, particularly in eutrophic fresh water bodies. During the summer of 2007, cyanoHABs threatened water quality of Taihu Lake in China and brought serious detrimental effects to the public life, economical and social development. The management of the polluted ecosystem has drawn increasing attention from both public and scientific communities [6,7]. In many lake restoration programs, reductions in nutrient loading, especially for phosphorus has resulted in reductions of cyanobacterial biomass [8]. However, long-term nutrient reduction can be costly and take many years for significant improvement of water quality [9]. The most direct strategies are through physical and chemical methods. A number of different devices have been deployed for the lake-scale collapse of gas vesicles in cyanobacteria, but the cost will rise with the pressure required [10]. Yellow loess [11] and clays [12] have been found to be effective on control of cyanobacterial bloom. However, they have side effects on bottom-dwelling organisms [13,14]. Application of algicides such as copper sulfate and hydrogen peroxide is not acceptable due to detrimental side effects on aquatic organisms and negative consequences for human health [15–18]. Biological methods have been developed to control cyanobacterial blooms [6,19,20]. Although many options are available to control cyanobacterial blooms, these methods require high-energy inputs and partly damage the aquatic environment [21]. Thus, there is still a need to select or develop new methods with fewer side effects on aquatic environments.

Interest in the application of white-rot fungi for the bioremediation of environmental pollutants is growing [22]. White-rot fungi can also decolorize textile dyes and remove complex-structured compounds by extracellular ligninolytic enzyme systems [23–27]. Some white-rot fungal species have shown potential as algal suppressors [28–30]. Additionally, they can degrade algal cells and their toxin microcystin-LR [31]. Among white-rot fungi, *Trametes versicolor* are considered as the most commonly used model organisms in biodegradation studies due to their good ligninolytic properties, fast growth potentials, and environmental-friendly nature [32,33]. Therefore, the application of *T. versicolor* for the bioremediation of cyanobacteria is expected to be relatively ecological.

In this study, an experiment was designed to study the possible biodegradability of the white-rot fungus *T. versicolor* F21a on cyanobacteria

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Table 1
Enzyme activity assayed in the two *Microcystis* spp. cultures, their active international unit (IU) definition, and references.

Enzyme	Substrate	IU definition	Method references
Cellulase	Sodium tylose (Sinopharm Chemical Reagent Co., Ltd)	1 μg glucose released min^{-1} ml^{-1} suspension	[60]
β-Glucosidase	p-Nitrophenyl-β-D-glucopyranoside (Sigma-Aldrich)	1 μmol PNP released h^{-1} ml^{-1} suspension	[61]
Protease	Caseins (Sinopharm Chemical Reagent Co., Ltd)	1 μg tyrosine released min^{-1} ml^{-1} suspension	[62]
ALP	Disodium phenyl phosphate (Sinopharm Chemical Reagent Co., Ltd)	1 mg phenol released h^{-1} ml^{-1} suspension	[63]
Laccase	ABTS (Bio Basic Inc.)	1 mol ABTS oxidized min^{-1} L^{-1} suspension	[64]
MnP	$MnSO_4$ (Sinopharm Chemical Reagent Co., Ltd)	1 μmol Mn^{2+} oxidized min^{-1} L^{-1} suspension	[65]

Microcystis spp., two toxic species in freshwater lakes [8]. Little is known about the dynamics of enzyme activities during fungus degrading cyanobacteria. Therefore, changes in the activities of extracellular enzymes involved in fungal metabolism were determined to investigate the possible mechanism of cyanobacterial degradation.

2. Materials and methods

2.1. Fungal strain and maintenance

The white-rot fungus *T. versicolor* F21a (F21a) with white hypha was isolated from the soils of Zijin Mountain (Nanjing, China). To identify the species of F21a, its 18S rRNA genes were amplified using a universal fungal primer pair ITS1F/ITS4 [34]. The nucleotide sequences of the isolates were analyzed with the BlastN search program from the National Center for Biotechnology Information (NCBI) website and deposited in GenBank under accession no. JF439512. This strain was maintained on potato dextrose agar (PDA) plates (200 g of potatoes; 20 g of dextrose; 20 g of agar and 1 l of distilled water) for 5 d, stored at 4 °C, and subcultured every month. A piece of round fungal mycelium from a PDA plate was inoculated in a 9 cm plate containing 15 ml of potato liquid medium and maintained at 28 °C. After 5 d of cultivation, mycelial pellicles in the plates were used for the experiments.

2.2. Microcystis strains and cultivation

The harmful cyanobacterial species, *Microcystis aeruginosa* FACHB-912 (FACHB-912) and *M. aeruginosa* FACHB-905 (FACHB-905), were provided by the Freshwater Algae Culture Collection (Wuhan, China). The cultures were maintained at 25 °C under a 12 h:12 h (L:D) cycle at approximately 90 μmol photons $m^{-2}\cdot s^{-1}$ [35]. *Microcystis* strains were then cultivated in a BG-11 medium [36] containing 150 mg of $NaNO_3$, 4 mg of $K_2HPO_4\cdot 3H_2O$, 7.5 mg of $MgSO_4\cdot 7H_2O$, 3.6 mg of $CaCl_2\cdot 2H_2O$, 0.6 mg of citric acid, 0.6 mg of ·ferric ammonium citrate, 0.1 mg of EDTA, 2 mg of Na_2CO_3, 0.1 ml of A_5 solution (H_3BO_3, 286 mg; $MnCl_2\cdot 4H_2O$, 186 mg; $ZnSO_4\cdot 7H_2O$, 22 mg; $Na_2MoO_4\cdot 2H_2O$, 39 mg; $CuSO_4\cdot 5H_2O$, 8 mg; $Co(NO_3)_2\cdot 6H_2O$, 5 mg in 100 ml of distilled water), and 99.9 ml of distilled water (pH 7.5). All chemicals used were of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd in China.

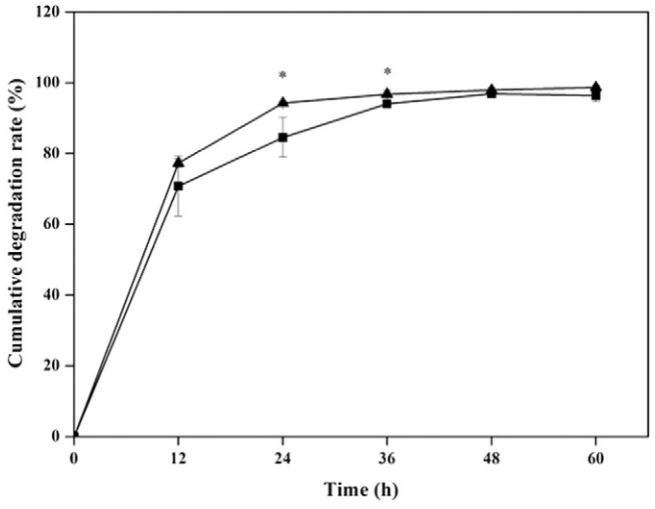

Fig. 1. Changes in the cumulative degradation rates of the two *Microcystis* spp. cultures. Symbols: ▲ *Microcystis aeruginosa* FACHB-912 incubation with *Trametes versicolor* F21a; ■ *Microcystis aeruginosa* FACHB-905 incubated with *Trametes versicolor* F21a. Significant differences between the two *Microcystis* strains are indicated by $^*P < 0.05$.

2.3. Experimental design

FACHB-912 and FACHB-905 cultures were centrifuged at 5000 rpm at 20 °C for 20 min separately, rinsed three times with BG-11 medium, and then resuspended in a fresh BG-11 medium. The densities of FACHB-912 culture and FACHB-905 culture were both adjusted to a concentration of 1×10^6 cells per ml. Four treatments were prepared on monocultures of FACHB-912 and FACHB-905: (1) FACHB-912 cultures: no mycelia pellicle addition; (2) FACHB-912&F21a: mycelial pellicle added to FACHB-912 cultures; (3) FACHB-905 cultures: no mycelia pellicle addition; (4) FACHB-905&F21a: mycelial pellicle added to FACHB-905 cultures. Additionally, two controls were conducted as: (1) BG-11 medium: no cyanobacterial cells or mycelia pellicle addition and (2) BG-11&F21a: mycelial pellicle added to the BG-11 medium. The initial dry weight of each mycelial pellicle was 35.46 ± 0.73 mg. Each 50 ml of the cultures was inoculated in an autoclaved flask and cultivated in a shaking incubator (27 °C, 120 rpm). All of the experiments were conducted in triplicate. The incubation period was 60 h. Cyanobacterial density, fungal biomass, and enzyme activities were monitored at 12 h intervals.

2.4. Degradation of F21a on Microcystis spp.

The cyanobacterial cell density was tested by determining the absorbance at 680 nm (OD_{680}) with a spectrophotometer (756MC, Shanghai Jinghua Science and Technology Instrument Co., Ltd.,

Table 2
Decomposition coefficients (κ, h^{-1}) of the two *Microcystis* spp. cultures.

Time (h)	FACHB-912	FACHB-905
12	0.1050 ± 0.0253^b	0.1235 ± 0.0045^a
24	0.0800 ± 0.0168^b	0.1190 ± 0.0033^a
36	0.0785 ± 0.0047^a	0.0960 ± 0.0081^a
48	0.0732 ± 0.0086^a	0.0816 ± 0.0075^a
60	0.0567 ± 0.0087^a	0.0717 ± 0.0059^a

Note: Data are presented as means ± SD, $n = 3$. Different letters denote significant differences ($P < 0.05$) between the two treatments. FACHB-912 corresponds to *Microcystis aeruginosa* FACHB-912 cultures incubated with *Trametes versicolor* F21a; and FACHB-905 corresponds to *Microcystis aeruginosa* FACHB-905 cultures incubated with *Trametes versicolor* F21a.