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Short communication

Inhibition of freshwater algal species by co-culture with two fungi

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ABSTRACT

Microorganisms have attracted worldwide attention as possible agents for the inhibition of water blooms. Algae can usually be inhibited and degraded directly by fungi. In this study, the effects of *Trichaptum abietinum* 1302BG and *Lopharia spadicea* on different freshwater algal species, namely, *Microcystis aeruginosa*, *Chlorella vulgaris*, *Glenodinium* sp., *Navicula* sp., *Cryptomonas ovata*, and *Euglena gracilis*, were detected. After 24 h, there was a significant inhibitory effect in all algal cultures with *T. abietinum* 1302BG, except *E. gracilis*, and all algal cultures with *L. spadicea*, except *Navicula* sp. and *E. gracilis*. The dried masses of two fungi increased while majority of the algal cells disappeared after 72 h of co-incubation with *M. aeruginosa*, *C. vulgaris*, *Glenodinium* sp., and *C. ovata*. Thus, the two fungi might inhibit the growth of different freshwater algal species and utilize the algal cells for their growth.

1. Introduction

In recent years, the occurrence of harmful algal blooms (HABs) has been reported worldwide. HABs affect public health, fishery industries, and freshwater ecosystems. Several physical and chemical methods have been developed to control algal blooms. Moreover, several strategies have been applied, such as the manipulation of physical sedimentation by flocculants and the application of chemical algicides and biological agents, in an effort to control the potentially devastating effects of HABs [1]. Previous studies have reported that bacteria [2,3] and cyanophages [4–7] are important algal lysing agents in many lakes. A number of algicidal bacteria have been isolated, and reports about their algicidal effects on *Anabaena cylindrica*, *Synechococcus cedorum*, *Nostoc* sp., *Phormidium tadzschicicum*, *Aphanizomenon flos-aquae*, *Anabaena circinalis*, *Nostoc ellipsosporum*, *Oscillatoria* spp., and *Microcystis* sp. have already been published [2,8–11]. Recently, certain species of fungi have shown promising potential as harmful algae suppressors in freshwater ecosystems. A previous study showed that the white rot fungi *Trichaptum abietinum* 1302BG and *Lopharia spadicea* inhibited the growth of *Microcystis aeruginosa* [12,13].

In the present study, the inhibitory abilities of the two white-rot fungi *T. abietinum* 1302BG and *L. spadicea* on different freshwater algae species were demonstrated.

2. Materials and methods

2.1. Algal strains and cultivation

The different algal species, namely, Cyanophyta (*Microcystis aeruginosa*), Chlorophyta (*Chlorella vulgaris*), Dinophyta (*Glenodinium* sp.), Bacillariophyta (*Navicula* sp.), Cryptophyta (*Cryptomonas ovata*), and Euglenophyta (*Euglena gracilis*), were provided by the Freshwater Algae Culture Collection of the Chinese Academy of Sciences, Wuhan, China.

The growth medium for *M. aeruginosa* and *C. vulgaris* was BG11. It was composed of 1.5 g $NaNO_3$, 40 mg K_2HPO_4, 75 mg $MgSO_4 \cdot 7H_2O$, 36 mg $CaCl_2 \cdot 2H_2O$, 58 mg $Na_2SiO_3 \cdot 9H_2O$, 6 mg citric acid, 6 mg ferric ammonium citrate, 1 mg EDTA, 20 mg Na_2CO_3, 1 mL A_5 solution, and 999 mL distilled water (pH 7.5). The A_5 solution comprised 286 mg H_3BO_3, 181 mg $MnCl_2 \cdot 4H_2O$, 22 mg $ZnSO_4 \cdot 7H_2O$, 7.9 mg $CuSO_4 \cdot 5H_2O$, 39 mg $Na_2MoO_4 \cdot 2H_2O$, 5 mg $Co(NO_3)_2 \cdot 6H_2O$, and 1000 mL distilled water.

The growth medium for *Glenodinium* sp. was D1. It was composed of 120 mg $NaNO_3$, 40 mg K_2HPO_4, 80 mg KH_2PO_4, 70 mg $MgSO_4 \cdot 7H_2O$, 20 mg $CaCl_2 \cdot 2H_2O$, 100 mg $Na_2SiO_3 \cdot 9H_2O$, 0.2 mg $MnSO_4$, 6 mg ferric citrate, 1 mL A_5 solution (286 mg H_3BO_3, 181 mg $MnCl_2 \cdot 4H_2O$, 22 mg $ZnSO_4 \cdot 7H_2O$, 7.9 mg $CuSO_4 \cdot 5H_2O$, 39 mg $Na_2MoO_4 \cdot 2H_2O$, 5 mg $Co(NO_3)_2$ $6H_2O$, and 1000 mL distilled water), and 20 mL soil extraction (200 g garden soil without a chemical fertilizer was added into the flask with 1000 mL distilled water and sealed with a ventilation plug; the solution was precipitated for 24 h after 3 h of boiling (repeated 3 times), filtered using paper filters, sterilized (121 °C, 20 min), and stored at 4 °C), and 999 mL distilled water.

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The growth medium for *Navicula* sp. was 119. It was composed of 50 mg $NaNO_3$, 15 mg $MgSO_4 \cdot 7H_2O$, 7 mg KH_2PO_4, 10 mg $CaCl_2 \cdot 2H_2O$, 5 mg $Na_2SiO_3 \cdot 9H_2O$, 10 mg $KHCO_3$, 5 mg Na_2CO_3, 0.2 mg MnSO4, 1 mL Trace mental solution (PIV, 750 mg Na_2EDTA, 41 mg $MnCl_2 \cdot 4H_2O$, 5 mg $ZnCl_2 \cdot 7H_2O$, 4 mg $Na_2MoO_4 \cdot 2H_2O$, 97 mg $FeCl_3 \cdot 6\ H_2O$, 2 mg $CoCl_2 \cdot 6\ H_2O$, and 1000 mL distilled water), 1 mL EDTA-Fe (0.901 g $FeCl_3 \cdot 6H_2O$ was resolved into 10 mL 1 N HCl and then 10 mL 0.1 N EDTA-Na_2 diluted to 1000 mL with distilled water was added), 30 mL soil extraction (200 g garden soil without a chemical fertilizer was added into the flask with 1000 mL distilled water and sealed with a ventilation plug; the solution was precipitated for 24 h after 3 h of boiling (repeated 3 times), filtered using paper filters, sterilized (121 °C, 20 min), and stored at 4 °C), and 998 mL distilled water.

The growth medium for *C. ovata* was AF-6. It was composed of 140 mg $NaNO_3$, 22 mg NH_4NO_3, 30 mg $MgSO_4 \cdot 7H_2O$, 5 mg K_2HPO_4, 10 mg KH_2PO_4, 10 mg $CaCl_2 \cdot 2H_2O$, 2 mg Fe-citrate, 2 mg citric acid, 2 μg Biotin, 10 μg Thiamine HCl, 1 μg Vitamin B6, 1 μg Vitamin B12, 0.4 g MES, 6 mL Trace mental solution (PIV), and 994 mL distilled water (pH 6.6). The PIV solution was composed of 750 mg Na_2EDTA, 41 mg $MnCl_2 \cdot 4H_2O$, 5 mg $ZnCl_2 \cdot 7H_2O$, 4 mg $Na_2MoO_4 \cdot 2H_2O$, 97 mg $FeCl_3 \cdot 6\ H_2O$, 2 mg $CoCl_2 \cdot 6\ H_2O$, and 1000 mL distilled water.

The growth medium for *E. gracilis* was HUT. It was composed of 20 mg K_2HPO_4, 600 mg Peptone, 25 mg $MgSO_4 \cdot 7H_2O$, 400 mg Yeast extract, 400 mg Sodium acetate, 40 mg Potassium citrate, 0.4 mg Vitamin B1, 0.5 μg Vitamin B12, and 1000 mL distilled water (pH 6.6).

All algal strains were conducted at 25 °C under a 12:12 h (Light: Dark) cycle at approximately 90 μmol photons $m^{-2}\ s^{-1}$. The flasks were shaken manually twice each day and rearranged randomly.

2.2. Fungal strains and maintenance

Two fungus strains, namely, TA-1302 and LS-1302, were isolated from the soils of bamboo forests (Hangzhou, China) and Qixia Mountain (Nanjing, China), respectively. The isolated strain was maintained on potato dextrose agar (PDA) plates for 7 days, stored at 4 °C, and sub-cultured every month. A piece of round fungal mycelia from a PDA solid-plate (2%) was inoculated into 9 cm diameter plates that contained 15 mL liquid potato dextrose broths (PDA) in a stationary position. After 7 days of cultivation, the mycelial pellicles were used in further experiments. All fungal isolates were kept at 4 °C after the initial screening for use in later experiments.

2.3. Inhibiting tests of different fungi on different algal species

The batch liquid tests were conducted in 250 mL Erlenmeyer flasks that contained 100 mL algal medium and a piece of mycelia pellicle. The dry weight of each inoculum was 71.03 ± 0.32 mg. The biomass of samples for different algal cultures was conducted at 0, 4, 8, 18, 24, 36, 48, and 72 h to evaluate the inhibitory effects of fungi on different algal species. The biomass in the algal samples was measured using a UV-2100 spectrophotometer at $\lambda = 680$ nm. Controls throughout the experiments were the same cultures as the testing groups, but without an inoculating mycelia pellicle. The inhibiting tests were conducted at 25 °C under a 12:12 h (Light:Dark) cycle at approximately 90 μmol photons $m^{-2}\ s^{-1}$ and 120 rpm. All experiments were conducted in triplicate.

2.4. Dry weight of the mycelial pellicle

The mycelial pellicle was filtered using polycarbonate filters (25 mm in diameter, 0.22 μm in mesh) at 72 h, rinsed with sterilized deionized water, and dried using a freeze–dry system (FreeZone-6, Labconco). The mass of each dried mycelial pellicle sample was measured before and after the inhibiting test.

2.5. Statistical analysis

All determinations were carried out in triplicate and mean values were presented. One-way ANOVA was conducted using SPSS PC + 13.

3. Results

The influences of TA-1302 and LS-1302 on the biomass of different freshwater algal species are shown in Fig. 1. The biomasses of the TA-1302 test cultures of (algal culture incubated with TA-1302) *M. aeruginosa*, *C. vulgaris*, *Navicula* sp., *C. ovata*, and *Glenodinium* sp. decreased very quickly within 18 h compared with those of the control (Fig. 1A, B, D–F). After 24 h, there were significant differences between these test cultures and their controls. However, the biomass of *E. gracilis* incubated with TA-1302 was significant higher than the control in 72 h (Fig. 1C).

The biomasses of the LS-1302 test cultures of (algal culture incubated with LS-1302) *M. aeruginosa*, *C. vulgaris*, *Navicula* sp., *C. ovata*, and *Glenodinium* sp. decreased very quickly within 18 h compared with the control (Fig. 1A, B, D–F). After 24 h, there were significant differences between these test cultures and their controls, except *Navicula* sp. The biomass of *Navicula* sp. incubated with LS-1302 increased significantly after 18 h. The biomass of *E. gracilis* incubated with LS-1302 in 72 h was also significantly higher than that of the control.

The mass of the mycelial pellicle (TA-1302 and LS-1302) co-incubated with different freshwater algal species is shown in Table 1. The mass of TA-1302 in different algal cultures of *M. aeruginosa*, *C. vulgaris*, *Glenodinium* sp., and *Navicula* sp. increased significantly after 72 h compared with that of BG11 (Table 1). There were significant differences between the mycelial pellicles of TA-1302 and BG11, except for the mycelial pellicles in the *Glenodinium* sp. and *E. gracilis* cultures. The mass of LS-1302 in different algal cultures of *M. aeruginosa*, *C. vulgaris*, *Glenodinium* sp., and *Navicula* sp. also increased significantly after 72 h compared with that of BG11 (Table 1). There were significant differences between mycelial pellicles of LS-1302 and BG11, except for the mycelial pellicles in the *Glenodinium* sp. and *E. gracilis* cultures.

4. Discussions

The two algicidal fungi (TA-1302 and LS-1302) belong to the order Polyporales of Basidiomycota. Species within the order Polyporales are saprotrophic fungi, and most of them are wood rotters. Many fungi that belong in this order have been investigated for the control or remediation of environmentally hazardous materials in soils or water bodies, such as polycyclic aromatic hydrocarbons, textile dye effluents, endocrine-disrupting compounds, and so on [14–20]. Some wood-decaying fungi also have algicidal abilities. The mechanism may be that the algal cells were encased with a mucous membrane secreted by the fungal mycelia, and finally degraded by the fungus directly [12]. During the degradation process, the residual algae could still grow in the culture. Therefore, the *Navicula* sp. presented an increment in the algal biomass after 18 h of incubation. Moreover, in the present work, the growth of freshwater algal species, namely, *M. aeruginosa*, *C. vulgaris*, *Navicula* sp., *C. ovata*, and *Glenodinium* sp. was significantly inhibited by TA-1302 and LS-1302, except for *E. gracilis*. Previous studies showed that Euglenophyta (*E. gracilis*) usually lived in ponds and pools, especially the water rich in organic matter. In some cases, they may ingest bacteria or other small organisms different from other algae species [21]. Therefore, *E. gracilis* may absorb some materials secreted by TA-1302 or LS-1302 for growth in the co-incubation culture. It is necessary for this phenomenon to be tested in future work.

Some bacteria and fungi have been reported to inhibit the growth of or degrade algae. Ahn et al. [22] reported that a *Bacillus subtilis* C1 culture containing 10 mg L^{-1} surfactin completely inhibited the growth