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RESEARCH PAPER

Allelopathic inhibitory effect of *Myriophyllum aquaticum* (Vell.) Verdc. on *Microcystis aeruginosa* and its physiological mechanism

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Abstract: Eutrophication and algal blooms are the most serious environmental problems in the world, and biological tools, especially the allelopathic inhibitory effects of aquatic macrophytes on phytoplankton growth have been receiving world-wide attention. In our experiments, the allelopathic inhibitory effect of *Myriophyllum aquaticum* culture water on *Microcystis aeruginosa* and its eco-physiological mechanism were investigated by initial addition assays and continuous addition assays. The results showed that the growth of *M. aeruginosa* was markedly inhibited by *M. aquaticum* culture water. Compared with initial addition assays, *M. aquaticum* exhibited stronger inhibitory potential on *M. aeruginosa* by continuous addition assays, indicating that allelopathic compounds might be excreted continuously by *M. aquaticum*, and the inhibitory effects would be cumulative. We also found that the relative content of chlorophyll a (Chl a), phycocyanin (PC) and allophycocyanin (APC) of *M. aeruginosa* decreased to 52.7%, 15.3% and 7.6% respectively after being treated by *M. aquaticum* culture water for 5 days, and phycobiliprotein (especially APC) decreased more than Chl a. These results suggest that the phycobiliprotein would be the target of allelopathic inhibition of *M. aquaticum* on *M. aeruginosa*, and a new macrophyte to control cyanobacterial blooms would be found.

Key Words: *Myriophyllum aquaticum* (Vell.) Verdc.; *Microcystis aeruginosa*; allelopathic inhibitory effects; photosynthetic system

In eutrophic water, rapid growth of cyanobacteria has induced an imbalance in the aquatic ecosystem and posed great threats to human health. Therefore, control and elimination of the cyanobacterial blooms have become a significant goal in environmental science. At present, several measures were conducted to control blue-green algal blooms, including physical methods (*e.g.*, mechanical algaecide), chemical ones (*e.g.*, adding algaecide or anti-algal flocculants) and biological ones (*e.g.*, introducing fish, cultivating aquatic plants and using algicidal bacteria). Biological technology has been widely accepted because of its higher environmental safety and relatively low operating cost[1]. Macrophytes were paid more attention because of their multifunction to restore and renew the damaged aquatic ecosystem, *e.g.*, secreting the anti-algal allelochemicals, removing nutrients from water and improving the aquatic landscape[1−5]. It is reported that macrophytes such as *M. spicatum*[5−10], *C. demersum*[5,11−14], *Chara*[15−18], *Eichhornia crassipes*[2,19,20], *Phragmitis communis Trin*[21,22] and *Acorus tatarinowii*[23,24] can inhibit growth of phytoplankton by its excretion. *M. aquaticum* of the genus *Myriophyllum* L. (Haloragaceae) was applied to the ecological restoration of the damaged aquatic ecosystem due to its considerable advantages, such as growing rapidly in eutrophic water, removing pollutants in the course of the development, and improving the aquatic landscape[4,25,26]. However, to date, the allelopathic effects and mechanisms of *M. aquaticum* on algae have apparently not been discussed.

At present, study on the allelopathic effects of macrophytes on algae has mainly focused on the following two aspects: (1) isolation and identification of the anti-algal allelopathic compounds from macrophytes leading to the artificial synthesis of the algaecide; (2) study on the eco-physiological mechanisms

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of the allelopathic effects of macrophytes on algae. For the latter, much evidence acquired by earlier research has suggested that some allelochemicals from macrophytes could inhibit algal growth or kill them through damaging algal ultra-structure[27,28], photosynthesis (such as destroying Chl a or photosystem II of algae and reducing their photosynthetic rate) and respiration of algae[6–8,15,16,27,28], or disturbing syntheses of some protein or their enzyme activity[6,7,20,28]. Generally, the great vitality of cyanobacteria is mainly attributed to their unique photosynthesis system[29–32]. However, there has been little knowledge regarding the mechanism of the effects of macrophytes on the photosynthesis system cyanobacteria.

The allelopathic effects of *M. aquaticum* on *M. aeruginosa* and the sensitive site of the photosynthetic system of *M. aeruginosa* are investigated with initial addition assays and continuous addition assays. In other words, the research focuses on two problems as follow: (1) Does *M. aquaticum* have an allelopathic effect on *M. aeruginosa* and how does it act? (2) If so, which part of the photosynthetic system is the most sensitive to the allelochemicals from *M. aquaticum*? The answers should provide a valuable evidence for the further comprehensive explanation of the allelopathy of aquatic ecosystems and its mechanism.

1 Materials and methods

1.1 Materials

M. aquaticum, after being collected from the wetland of Dianchi Lake, Yunnan of China, were washed with tap water, and then were cultivated in aerated water. *M. aeruginosa* (FACHB 905 stain) and its culture medium were provided by the Institute of Hydrobiology of the Chinese Academy of Sciences. The algae were acclimated firstly for a week in the updated HGZ-145 (temperature 25°C±1°C), and then were cultivated in the culture medium up to their exponential growth phase. All these operations were conducted under sterile conditions.

1.2 Components of the updated HGZ-145 culture medium

The updated HGZ -145 culture medium in the research was provided following the Table 1.

Table 1 Components of the updated HGZ -145 culture medium

Major nutrient	Content (g/L)	Trace element	Content (g/L)
$NaNO_3$	0.2	HBO_3	0.00286
KNO_3	0.051	$MnCl_2·4H_2O$	0.00181
K_2HPO	0.049	$ZnSO_4·7H_2O$	0.000222
$MgSO_4·7H_2O$	0.075	$Na_2Mo_4·2H_2O$	0.000391
Na_2CO_3	0.02	$CuSO_4·5H_2O$	0.000079
$Ca(NO_3)_2·4H_2O$	0.059	Fe-EDTA	0.000932
NH_4Cl(NH_4Cl- EDTA)	0.0391		

Note: NH_4Cl (NH_4Cl- EDTA) and Fe-EDTA were prepared following the 24th Reference.

1.3 *M. aquaticum* cultivation and preparation of its culture water

The aquarium, with 7000 ml distilled water (34 cm × 15 cm × 24 cm), was wrapped by a piece of white paper to prevent the photodecomposition of some allelochemicals from *M. aquaticum* in bright strong light[20]. About 110 g of *M. aquaticum* was planted for 10 d in the aquarium located outdoors in a semi-enclosed shed (the whole experiment period was from August 2006 to February 2007). Then, 1000 ml of culture water, taken from the aquarium, filtrated with a 0.45 μm ultrafiltrate membrane (Millipore, USA) and added with updated HGZ-145 medium, would be used in the assays. During the continuous addition assays, 100 ml of culture water was prepared daily for adding to the treatments, while the control would add the HGZ-145 culture medium.

1.4 *M. aeruginosa* cultivation and measure of several indices

1.4.1 Initial addition assays

10 ml of *M. aeruginosa* solution was inoculated with a 500 ml Erlenmeyer flask containing 150 ml of the culture water medium (as in Section 1.3), while the control was prepared by inoculating algae into the HGZ-145 medium. Then both of them were cultivated for 25–30 d under an artificial climate box (SHH-500GS, Chongqing, China, at a temperature of 25 °C±1°C, a light density of 2500 lux and 12 h light/8 h darkness). The flasks would be moved frequently and shaken slightly 4 times per day. The growth curve of algae would be drawn by daily measuring the value of optical density of the algae sample at 663 nm wavelength (*i.e.*, OD_{663}). The treatment and the control were both performed on 3 samples. All the processes of these operations were conducted in sterile conditions.

1.4.2 Continuous addition assays

10 ml of algae was inoculated to a 250 ml Erlenmeyer flask containing 100 ml of the culture water medium (as in Section 1.3), and the remaining operations were the same as for the initial addition. But when the several indices of algae samples were to be determined (as in Section 1.4.3), the corresponding volume of the medium was supplied every day.

1.4.3 Monitoring indices and determination

The growth curve of *M. aeruginosa* was determined by measuring the OD_{663} value of the algae samples with a spectrophotometer (UV755B, Shanghai of China).

The calculation of the *IR* (Inhibition Ratio) followed Ref.[21]. The function is $IR(100\%) = (1 - N_t / M_t) \times 100$, where N_t and M_t indicate the OD_{663} value of *M. aeruginosa* of the treatment and control at different times, respectively.

The relative Chl a (η) contents were measured by following Ref.[32]. The function is $\eta(100\%) = \psi_t / \varphi_t \times 100$, where