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Growth inhibition and oxidative damage of *Microcystis aeruginosa* induced by crude extract of *Sagittaria trifolia* tubers

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ABSTRACT

Aquatic macrophytes are considered to be promising in controlling harmful cyanobacterial blooms. In this research, an aqueous extract of *Sagittaria trifolia* tubers was prepared to study its inhibitory effect on *Microcystis aeruginosa* in the laboratory. Several physiological indices of *M. aeruginosa*, in response to the environmental stress, were analyzed. Results showed that *S. trifolia* tuber aqueous extract significantly inhibited the growth of *M. aeruginosa* in a concentration-dependent way. The highest inhibition rate reached 90% after 6 day treatment. The *Chlorophyll-a* concentration of *M. aeruginosa* cells decreased from 343.1 to 314.2 μg/L in the treatment group. The activities of superoxide dismutase and peroxidase and the content of reduced glutathione in *M. aeruginosa* cells initially increased as a response to the oxidative stress posed by *S. trifolia* tuber aqueous extract, but then decreased as time prolonged. The lipid peroxidation damage of the cyanobacterial cell membranes was reflected by the malondialdehyde level, which was notably higher in the treatment group compared with the controls. It was concluded that the oxidative damage of *M. aeruginosa* induced by *S. trifolia* tuber aqueous extract might be one of the mechanisms for the inhibitory effects.

Introduction

Outbreaks of cyanobacterial blooms have been indeed increasing in frequency and geographical distribution in the last decades, mostly due to climate changes (Paerl, 2009; Paerl, 2012). Large-scale cyanobacterial blooms degrade water quality and pose serious threats to aquatic organisms and even human health (Paerl et al., 2001). *Microcystis aeruginosa* is one of the representative species of bloom-forming cyanobacterium that occur in freshwater cyanobacterial blooms. Microcystins, a kind of cyanotoxin produced by toxic strains of *M. aeruginosa*, can be very harmful to the human liver through the food chain due to its hepatotoxicity (Mankiewicz et al., 2003). Therefore, it is of great importance to suppress/inhibit the growth of *M. aeruginosa* in eutrophic waters.

Compared with physical methods (*e.g.*, ultraviolet irradiation (Sakai et al., 2007)) or chemical methods (*e.g.*, nitrite (Chen et al., 2011)), the utilization of biological treatment in cyanobacterium control is a relatively cost-effective and environment-friendly approach. Research using aquatic plants and their allelopathic

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effects in *M. aeruginosa* control has been extensively carried on in recent years (Chang et al., 2012; Zhang et al., 2009). *Myriophyllum spicatum* was shown to be one of the most effective macrophyte species, secreting allelochemicals such as pyrogallic acid and gallic acid to inhibit the growth of cyanobacteria (Nakai et al., 2000; Zhu et al., 2010). Chen et al. (2012) investigated the effects of eight species of aquatic macrophytes on *M. aeruginosa* growth and demonstrated that leaves of *Nymphacea tetragona*, *Typha orientalis*, *Nelumbo nucifera* and *Iris wilsonii* were the most potent tissues to inhibit its growth.

The main experimental approaches for studying how aquatic macrophytes affect the growth of phytoplankton can be concluded as follows: coexistence experiments, plant homogenates or extracts, culture filtrates, active compounds extracted from the culture filtrate, and dialysis bag experiments (Gross et al., 2007). Previous studies found that coexistence with *Lemna japonica* (Jang et al., 2007), exudates from *Stratiotes aloides* (Mulderij et al., 2005), decoction of *Radix Astragali* (Yan et al., 2011), essential oils from *Ceratophyllum demersum* and *Vallisneria spiralis* (Xian et al., 2006), and culture water of *Myriophyllum aquaticum* (Wu et al., 2008) all showed inhibitory effects on the growth of *M. aeruginosa*. In addition, allelochemicals from aquatic macrophytes, such as N,N-dimethyl-3-amino-methylindole (gramine) (Hong et al., 2009) and ethyl 2-methyl acetoacetate (EMA) isolated from *Phragmites communis* (Li and Hu 2005; Hong et al., 2008a), have been reported to be useful alternatives to inhibit the growth of *M. aeruginosa*. All those studies mentioned above suggested that aquatic macrophytes might have the ability to control cyanobacterial growth through allelopathy. In addition, other effects may also play a role, such as competing with harmful cyanobacteria for light and nutrients.

Damage in the electron transfer system can result in the formation of reactive oxygen species (ROS), such as superoxide radical (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH), and may then cause peroxidation damage to both plasmalemma and intracellular membranes, finally leading to cell dysfunction and death (Scandalios 1993; Thannickal and Fanburg 2000). Under normal conditions, cells have specific antioxidant protective processes to combat the danger posed by ROS to a certain extent (Mallick and Mohn 2000) so that living cells can maintain a dynamic equilibrium between ROS generation and removal. But excessive radicals, if not eliminated in a timely fashion, may finally lead to cell damage and death. It was reported that allelochemicals from plants could induce ROS production and then lead to oxidant damage in *M. aeruginosa* cells (Hong et al., 2008b; Wang et al., 2011; Zhang et al., 2011a).

Sagittaria trifolia (also called *Arrowhead* due to the shape of its leaves) is one of the main emergent macrophytes and is widely spread in most parts of China. The edible tubers of *S. trifolia* have long been used as vegetables and traditional Chinese medicines in China. Works have reported that *Sagittaria* can absorb nitrogen and phosphorus in eutrophic water and show good effects in water purification (Li et al., 2009). However, to our knowledge, there is little information available about the inhibitory effects of *S. trifolia* on cyanobacterial growth. The use of plant extracts has been considered to be one of the most common experimental approaches for phytoplankton growth inhibition by macrophytes (Hilt and Gross 2008). The research method in the current study was designed after several kinds of pre-experiments involving *S. trifolia* leaf aqueous extract, *S. trifolia* root aqueous extract, and *S. trifolia* planting water. According to the comparison of experimental results, we found that the *S. trifolia* tuber aqueous extract was the most effective material for use in inhibitory experiments. Owing to their large biomass and widespread occurrence, the tuber of *S. trifolia* was chosen as the active inhibition material in our experiment and its aqueous extract was prepared. The purpose of our present work is to investigate the inhibitory effect of *S. trifolia* tuber aqueous extract on *M. aeruginosa* and to assess the extract-induced oxidant damage on *M. aeruginosa* cells by measuring several indices, including superoxide dismutase (SOD) activity, peroxidase (POD) activity, glutathione (GSH) content and malondialdehyde (MDA) level, to elucidate the potential anti- cyanobacterial mechanism.

1. Materials and methods

1.1. Materials and culture conditions

The tubers of *S. trifolia* were purchased from a farm in Huai'an City, Jiangsu Province, and stored in plastic buckets with moist soil at room temperature (about 20°C) before extraction. The cyanobacterium species *M. aeruginosa* was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Then it was cultured in autoclaved MA medium containing (in mg/L): $NaNO_3$ 50, KNO_3 100, $Ca(NO_3)_2{\cdot}4H_2O$ 50, Na_2SO_4 40, $MgCl_2{\cdot}6H_2O$ 50, β-sodium glycerophosphate 100, Na_2EDTA 5, $FeCl_3{\cdot}6H_2O$ 0.5, $MnCl_2{\cdot}4H_2O$ 5, $ZnCl_2{\cdot}7H_2O$ 5, $CoCl_2{\cdot}6H_2O$ 5, $Na_2MoO_4 \cdot 2H_2O$ 0.8, H_3BO_3 20, Bicine 500, under an illumination intensity of 4,000 lx and a light/dark regime of 12:12 hr at 25°C. The culture flasks were placed in a shaking incubator (Digital temperature water bath thermostatic oscillator, Changzhou Putian Instrument Manufacturing Co., Ltd., China) and shaken for 3 times during the light cultural period per day, each time lasting about 1 min.

1.2. Preparation of *S. trifolia* tuber aqueous extract

Selected *S. trifolia* tubers were washed in flowing water and then rinsed by ultrapure water three times, to remove debris and attached microorganisms as much as possible. The cleaned tubers were dried in a drying oven at 50°C to constant weight. The dehydrated samples were then cut into small pieces, powdered and mixed with ultrapure water (1:40 *w/v*). After boiling in covered flasks for 40 min (60°C, 40 min), the solution was filtered with filter paper to remove tuber residues. All the flasks containing the *S. trifolia* tuber aqueous extract were sterilized at 121°C for 20 min. The filtrate was utilized as the cyanobacteria-inhibiting aqueous extract in our experiment and kept at 4°C before use.

1.3. Experimental design

To study the inhibitory effects of different concentrations of *S. trifolia* aqueous extract on the growth of *M. aeruginosa*, conical flasks (500 mL) with cotton plugs were prepared and divided into 6 experimental groups. Each group of flasks contained 200 mL MA medium with different proportions of ultrapure