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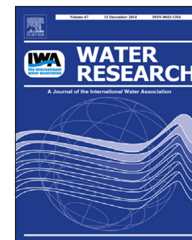

Extracellular polymeric substances buffer against the biocidal effect of H_2O_2 on the bloom-forming cyanobacterium *Microcystis aeruginosa*

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ABSTRACT

H_2O_2 is an emerging biocide for bloom-forming cyanobacteria. It is important to investigate the H_2O_2 scavenging ability of extracellular polymeric substances (EPS) of cyanobacteria because EPS with strong antioxidant activity may "waste" considerable amounts of H_2O_2 before it kills the cells. In this study, the buffering capacity against H_2O_2 of EPS from the bloom-forming cyanobacterium *Microcystis aeruginosa* was investigated. IC_{50} values for the ability of EPS and vitamin C (VC) to scavenge 50% of the initial H_2O_2 concentration were 0.097 and 0.28 mg mL^{-1}, respectively, indicating the higher H_2O_2 scavenging activity of EPS than VC. Both proteins and polysaccharides are significantly decomposed by H_2O_2 and the polysaccharides were more readily decomposed than proteins. H_2O_2 consumed by the EPS accounted for 50% of the total amount of H_2O_2 consumed by the cells. Cell growth and photosynthesis were reduced more for EPS-free cells than EPS coated cells when the cells were treated with 0.1 or 0.2 mg mL^{-1} H_2O_2, and the maximum photochemical efficiency Fv/Fm of EPS coated cells recovered to higher values than EPS-free cells. Concentrations of H_2O_2 above 0.3 mg mL^{-1} completely inhibited photosynthesis and no recovery was observed for both EPS-free and EPS coated cells. This shows that EPS has some buffering capacity against the killing effect of H_2O_2 on cyanobacterial cells. Such a strong H_2O_2 scavenging ability of EPS is not favorable for killing bloom-forming cyanobacteria. The high H_2O_2 scavenging capacity means considerable amounts of H_2O_2 have to be used to break through the EPS barrier before H_2O_2 exerts any killing effects on the cells. It is therefore necessary to determine the H_2O_2 scavenging capacity of the EPS of various bloom-forming cyanobacteria so that the cost-effective amount of H_2O_2 needed to be used for killing the cyanobacteria can be estimated.

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1. Introduction

The cyanobacterial blooms of freshwater lakes are a great ecological and human health risk due to the production of toxins such as microcystins and anatoxins (El-Shehawy et al., 2012; Horst et al., 2014). Exposure to cyanobacterial toxins may cause acute or chronic toxicity to neurosystems and many organs including the liver (Sivonen and Jones, 1999). Oxidation of the cyanobacteria cells by oxidants is one of the common ways to inhibit growth of harmful cyanobacteria (Matthijs et al., 2012; Rajasekhar et al., 2012). In comparison with O_3 and ClO_2^-, H_2O_2 is an emerging and inexpensive but effective biocide (Xu et al., 2007; Matthijs et al., 2012; Fan et al., 2013). A number of studies show that H_2O_2 can effectively kill various bloom-forming cyanobacteria (Drábková et al., 2007; Barrington and Ghadouani, 2008; Matthijs et al., 2012; Qian et al., 2010, 2012). The physiological processes inhibited by H_2O_2 include photosynthesis, antioxidant systems, synthesis of pigments and circadian rhythms (Hoeger et al., 2002; Barrington and Ghadouani, 2008; Qian et al., 2010, 2012).

Cyanobacteria are able to produce extracellular polymeric substances (EPS) which are mainly composed of polysaccharides and proteins (Wingender et al., 1999; Pan et al., 2012; Zhang et al., 2013; Pivokonsky et al., 2014). Some of the EPS are loosely attached to the cell surface but other parts can be tightly bound to the cell wall (Pan et al., 2010a, 2010b). The EPS physically serves as a barrier between the cell and the ambient environment (Pan, 2010). Owing to their strong ability to bind toxic pollutants, water holding capacity and antioxidant activity, they play a key role in buffering against adverse effects of various environmental stressors (Shepherd and Beilby, 1999; Pan, 2010). EPS isolated from bacteria *Pseudomonas* PF-6 (Ye et al., 2012) and *Bacillus megaterium* RB-05 (Chowdhury et al., 2011) have a stronger antioxidation ability to hydroxyl free radicals and the superoxide anion compared to the typical antioxidant, vitamin C (VC). The antioxidant activity, in the sense of physiological health of bacteria, makes EPS an indispensable protective buffering zone against damage from ROS (reactive oxygen species). On the other hand, from the perspective of killing toxic bloom-forming cyanobacteria, such strong ROS scavenging capacity of cyanobacteria implies that a high proportion of H_2O_2 would be consumed by the EPS before it can exert killing activity on the cell. In other words, the massive EPS around the cells can significantly reduce the killing effect of H_2O_2. Therefore, it is of importance to know how much H_2O_2 would be consumed when H_2O_2 is applied to kill harmful cyanobacteria. However, no information on this is currently available.

In the present study, the influence of EPS on the toxic effects of H_2O_2 on photosynthesis of *Microcystis aeruginosa*, a common bloom-forming cyanobacterium, was studied. The H_2O_2 scavenging ability of EPS from *M. aeruginosa* was quantitatively examined using VC as a reference.

2. Materials and methods

2.1. Cultivation of cyanobacterium

The cyanobacterium *M. aeruginosa* (FACH#905) was purchased from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China), and cultured in BG-11 medium (Stanier et al., 1971) at 30 °C under 50 μmol photons m^{-2} s^{-1} PAR with a 12:12 h light–dark cycle. Cyanobacterial cells in the exponential growth phase were used for extraction of EPS and physiological tests.

2.2. Effects of EPS on the killing activity of H_2O_2 on M. aeruginosa

The effect of H_2O_2 on the photosynthesis of *M. aeruginosa* was comparatively assessed in the absence and presence of EPS. In order to remove or extract EPS from the cells, the cell suspension was irradiated with 200 w ultrasound (HU20500B, Shanghai) for 20 min and then centrifuged at 14,000 r min^{-1} for 20 min (Pan et al., 2010a). The supernatant was collected as the raw EPS solution and the residual cells were considered as EPS free cells (herein defined as −EPS cells. The −EPS cells were checked by the Alcian blue 8GX staining method. 0.05% Alcian blue solution was prepared by dissolving Alcian blue 8GX of analytical grade in 3% acetic acid solution at pH 2–3. After 30 min of staining with Alcian blue solution, EPS on surfaces of cells was checked with a light microscope. If the cells were not stained with Alcian blue, this indicated that the EPS was successfully removed from the cells (Shepherd and Beilby, 1999; Böckelmann et al., 2003; Kallmeyer et al., 2008). The Alcian blue 8GX staining results showed that most EPS on the surface of the *M. aeruginosa* cells was successfully removed by the ultrasound-centrifugation extraction (Fig. S1).

In addition, in order to assess the effect of EPS extraction on the physiological status of cells, the maximum photochemical efficiency of photosystem II (Fv/Fm), which is a reliable indicator for photosynthesis of cyanobacteria, of cells before and after extraction of EPS was monitored using a dual-modulation fluorometer (FL-3500, PSI, Brno, CZ) (Pan et al., 2008, 2009). The cells were dark-adapted for 5 min before chlorophyll fluorescence testing (Pan et al., 2008). It was found that Fv/Fm was kept at relatively constant values of about 0.34 after EPS extraction, a little lower than the original value (0.35), indicating that the physiological status of the cells was not adversely affected by EPS extraction (Table S1).

The original cyanobacterial cells without EPS extraction, i.e., eps-coated cells (herein defined as +EPS cells) and −EPS cells were separately incubated in BG-11 medium containing various concentrations (0, 0.4, 0.8, 1.2 and 1.6 mg L^{-1}) of H_2O_2. The optical density at 668 nm (OD_{668}), chlorophyll *a* content and Fv/Fm of the cells untreated and treated with various H_2O_2 concentrations were measured at different time intervals. The Chl *a* content was determined by the colorimetric method (Arnon, 1949). Three mL of cell suspension was centrifuged at 8000 rpm for 5 min. The residual cells were extracted with 3 mL of 80% acetone at 4 °C in the dark for 24 h and then centrifuged at 8000 rpm for 5 min. The absorbance at 663 nm and 645 nm of the acetone extract was recorded with 80% acetone being used as the reference blank using a spectrophotometer (Unico 2000, Shanghai, China). Chl *a* content was estimated using the following formula: Chl *a* (mg L^{-1}) $= 12.7 \times A_{663} - 2.69 \times A_{645}$ (Arnon, 1949).

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