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Growth, physiochemical and antioxidant responses of overwintering benthic cyanobacteria to hydrogen peroxide☆

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The recruitment of overwintering benthic cyanobacteria from the sediment surface is important for the development of cyanobacterial blooms during warm spring seasons. Thus, controlling the growth of cyanobacteria at the benthic stage to inhibit their recruitment is vital to control or delay the formation of summer blooms. In this study, overwintering benthic cyanobacteria were exposed to ascending hydrogen peroxide (H_2O_2) concentrations (0, 1, 5, and 20 mg/L) in a simulated overwintering environment. Photosynthetic pigments, physiochemical features, and antioxidant responses were evaluated to determine the inhibitory effects of H_2O_2 on the growth of benthic cyanobacteria and to identify the potential mechanisms thereof. These H_2O_2-treated cyanobacteria were then collected through filtration and transferred to an optimum environment to evaluate their recovery capacity. The results showed that chlorophyll *a* and phycocyanin contents, photosynthetic yield, and esterase activity decreased significantly in H_2O_2 treated groups compared to the control. The activities of superoxide dismutase (SOD) and catalase (CAT) in benthic cyanobacteria were inhibited after 72 h exposure to H_2O_2, while the malondialdehyde (MDA) contents were stimulated at the same time. These results indicate that H_2O_2 can inhibit the growth of benthic cyanobacteria, and H_2O_2-induced oxidative damage might be one of the mechanisms involved. The recovery experiment showed that the impairment of benthic cyanobacteria was temporary at a low dose of 1 mg/L H_2O_2, but permanent damage was induced when H_2O_2 concentrations were increased to 5 and 20 mg/L. Overall, our results highlight that H_2O_2 is a potential cyanobacteria inhibitor and can be used to decreasing the biomass of overwintering cyanobacteria, and could further control the intensity of cyanobacteria during the growth seasons.

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1. Introduction

Cyanobacterial blooms, frequently implicated in the eutrophication of aquatic ecosystems, have become a considerable threat to the quality of surface waters, thereby limiting their potential uses, such as for drinking water, recreation, fishing, and, more fundamentally, ecological function (Paerl and Huisman, 2008). Numerous efforts involving the reduction of nutrients, chemical algicides, physical measures and biological approaches have been made to suppress populations of cyanobacteria and to improve the value of water resources (Daniel and Blahoslav, 2011). However, remediation measures are not always entirely satisfactory, as most control strategies are implemented after the outbreak of algae blooms (Jia et al., 2014), which may incur tremendous costs and lead to negative consequences such as microcystin release (Fan et al., 2013). Moreover, post-algal bloom control methods also neglect the annual life cycle of cyanobacteria, including the planktonic and benthic resting stages (Kong and Gao, 2005).

Microcystis, the most widely distributed cyanobacteria species in freshwater ecosystems (Shang et al., 2015), can sink to the sediment surface to overwinter in the form of vegetative colonies, and then repopulate the water by passive or active processes when environmental conditions are favorable (Kong and Gao, 2005). Previous studies suggested that the recruitment of benthic cyanobacteria may be important for the pelagic population and may even be responsible for bloom formation in summer (Reynolds et al., 1981; Annika et al., 2003). About 50% of the *Microcystis* colonies

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that were initially abundant in the surface sediments were recruited to the water column in a shallow bay (Anna and Prter, 2003), and decreasing their abundance would reduce the summer bloom by more than 64% (Jolanda et al., 2005). Thus, using an effective measure to reduce the biomass of benthic cyanobacteria may be a useful management strategy to suppress or at least delay cyanobacterial blooms in summer (Jolanda et al., 2005). It may not only reduce the biomass of cyanobacteria, but also eliminate the "seed bank" of cyanobacteria, and thus may contribute to longer term effectiveness compared to those remediation measures implemented after the outbreak of cyanobacteria (Fan et al., 2013; Lu et al., 2015).

Among all these remediation strategies, chemical algicide is regarded as an economical and effective way to inhibit the growth of target algae (Jia et al., 2014) although some drawbacks have been confirmed after using them (Daniel and Blahoslav, 2011). For example, previous studies demonstrated that 0.2 mg/L copper could successfully decrease the biomass of algae, but could be lethal to daphnias (Daniel and Blahoslav, 2011). However, recent studies demonstrated that hydrogen peroxide (H_2O_2) should be a promising chemical algicide as it may selectively inhibit the growth of cyanobacteria (Qian et al., 2010) and degrade into water and oxygen in a few hours or days without the production of persistent toxic chemicals. In fact, the H_2O_2 concentrations required to inhibit the growth of cyanobacteria were ten times lower than those required to inhibit the growth of green algae, since the photosynthetic system of cyanobacteria was more sensitive than that of green algae (Drábková et al., 2007a).

The addition of H_2O_2 can create oxidative stress through increased production of reactive oxygen species (ROS). Algae can survive under certain oxidative conditions, by increasing antioxidant defenses, notably enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Liu et al., 2012). Generally, stress-tolerant species have a more effective defense system against ROS than stress-susceptible species. However, when ROS concentrations reach relatively high levels, the capacity of enzymatic and non-enzymatic antioxidant systems can be completely destroyed, causing cell death (Dummermuth et al., 2003). In fact, the toxicity of H_2O_2 on algae is affected by several factors such as light intensity, temperature and algae species (Drábková et al., 2007b). Thus, we wondered if the growth of benthic cyanobacteria could be inhibited by H_2O_2 at their benthic resting stage under conditions of low light intensity and temperature. Moreover, understanding the responses of benthic cyanobacteria to hydrogen peroxide under these situations is essential for eliminating them in winter and then reducing the extent of the cyanobacteria bloom in summer. In the present study, overwintering benthic cyanobacteria were collected and exposed to ascending hydrogen peroxide concentrations under low light and temperature conditions. We analyzed the pigment contents, and the biochemical and physiological characteristics of benthic cyanobacteria between different treatments, to determine the inhibitory effects of H_2O_2 on the growth of benthic cyanobacteria and to identify its potential mechanisms. A simulated recovery experiment was also conducted to study the recovery capability of benthic cyanobacteria after H_2O_2 treatment, for evaluating the further control of cyanobacteria intensity during the growth seasons.

2. Materials and methods

2.1. Study site and algal material

Lake Chaohu, a shallow and turbid lake, is the fifth largest freshwater lake in China, with a surface area of 760 km^2 and a mean depth of 3.06 m. It is a severely eutrophied lake with frequent cyanobacterial blooms during summer: in recent decades these have been dominated by *Microcystis* and *Anabaena* (Shang et al., 2015). Sediments were sampled on December 20, 2015 using a cylindrical sampler at the center of Lake Chaohu (117°30′53.74″E, 31°30′53.25″N), where lacustrine sediment accumulates. Samples of the upper 0–2 cm of the surface layer sediments were removed and stored in bottles (50 L) during transportation to the laboratory. In total, about 50 L of lake water was collected at the site.

All the sediment samples were diluted (1/10) with distilled water and filtered through a 63 μm mesh (thus retaining the algal colonies) (Tsujimura et al., 2000). The algal colonies were separated from other particles on the filter with a pipette (Delphine et al., 2004), and then placed in an opaque beaker containing 400 mL of filtered lake water at a temperature of 8 °C under low light conditions until our experiment began.

2.2. Experimental procedures

Benthic cyanobacteria were put into 12 glass beakers (1000 mL) and then diluted with filtered lake water (Whatman GF/C, 600 mL) until the final concentration of chlorophyll *a* was the same. Then, H_2O_2 was added to these beakers to reach the final concentrations of 0, 1, 5, and 20 mg/L; the beakers without any H_2O_2 were used as the control, and each concentration was used in triplicate. The beakers were cultured at 8 ± 1 °C under 10 μmol photons m^{-2} s^{-1} of cold fluorescent light at an 8:16 h light:dark cycle for three days to simulate the *in situ* conditions at the bottom of Lake Chaohu in winter. Water samples were collected from each beaker to determine the biomass of algae and selected physiological and biochemical traits at 0, 12, 24, 48 and 72 h. After 72 h treatments with various H_2O_2 concentrations, benthic cyanobacteria were collected by filtration through a 63 mesh, and were then incubated in 250 mL flasks containing 100 mL sterilized BG11 medium. Then, the recovery experiment was performed under an irradiance of 40 μmol photons m^{-2} s^{-1} with a light:dark period of 12:12 h at 25 ± 1 °C. After seven days incubation, water samples were harvested to analyze the contents of chlorophyll *a* and phycocyanin.

2.3. Analytical methods

2.3.1. Measurement of chlorophyll a and phycocyanin

In this study, contents of chlorophyll *a* and phycocyanin in water samples were determined as a measure of the algal biomass (Cao et al., 2005). Each 10 mL water sample was filtered through glass microfiber filters (Whatman, GF/C) to determine the contents of chlorophyll *a* and phycocyanin. The membranes were ground into a homogenous matter, and then extracted with 90% acetone (for chlorophyll *a*) and 0.05 mol/L Tris-HCl (pH 7.0) (for phycocyanin) in darkness at 4 °C for 8 h. After centrifugation at 6000 rpm for 10 min at 4 °C, the supernatant was used to determine the contents of chlorophyll *a* and phycocyanin with a fluorescence spectrophotometer (Cao et al., 2005).

2.3.2. Measurement of photosynthetic capacity

Photosynthetic capacity was measured by phyto-PAM (Walz, Germany) after a 5 mL water sample was adapted to darkness for 15 min. The maximum optical quantum yield of PS II (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$, where F_0 and F_m indicated the minimum and maximum fluorescence light yields in a saturating light pulse after dark adaptation, respectively (Yang et al., 2015). The photosynthetic inhibition rate was calculated using: Inhibition (%) = $(1 - N/N_0) \times 100$, where N and N_0 are the maximum quantum yields in H_2O_2-treated groups and their corresponding control groups, respectively.

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