Environmental Science and Pollution Research (2023) 30:60654–60662
https://doi.org/10.1007/s11356-023-26840-6

RESEARCH ARTICLE

Biocidal H_2O_2 treatment emphasizes the crucial role of cyanobacterial extracellular polysaccharides against external strong oxidative stress

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Received: 27 September 2022 / Accepted: 3 April 2023 / Published online: 11 April 2023

Abstract

A biocidal level of hydrogen peroxide (H_2O_2), far beyond the natural level, is widely used to control bloom-forming cyanobacteria in freshwater. The extracellular polymeric substance of these cyanobacteria is a key factor in determining the applied H_2O_2 dosage. The exopolysaccharide (EPS) in the extracellular polymer shows H_2O_2 scavenging capability. However, the scavenging capabilities of EPSs from other cyanobacteria against biocidal levels of H_2O_2 as well as their protective roles against cyanobacterial cells are not well known. In this study, we used four nonbloom-forming cyanobacteria as target organisms, two with rich EPS envelopes (EPS-rich strains) and two with thin EPS envelopes (EPS-thin strains), to assess the roles of EPS. It was found that the two EPS-rich strains were much more tolerant to a high dose of exogenous H_2O_2 than the two EPS-thin strains. The EPSs extracted from the four strains exhibited similar but rapid H_2O_2 scavenging activity. Additionally, the EPSs from the EPS-rich strains could improve the tolerance of the EPS-thin strains to H_2O_2 stress, implying potentially nonselective protection against oxidative stress. In addition, all the cell lysates of the four strains showed H_2O_2 decomposition ability, with the efficiency being slightly different between the two types of strains. This study suggests that cyanobacterial EPS plays a generally crucial role against external strong oxidative stress and may provide a useful reference for the application of H_2O_2 in environmental management.

Keywords Cyanobacteria · Exopolysaccharide · Hydrogen peroxide · Oxidative stress · Environmental management

Introduction

Hydrogen peroxide (H_2O_2) is the simplest peroxide and is also a powerful oxidizing agent. This molecule can occur naturally in aquatic environments from dissolved chromophoric organic materials exposed to sunlight (Clark et al. 2014). However, the major natural source of H_2O_2 is biologically generated by phytoplankton (Diaz and Plummer 2018). The natural H_2O_2 concentrations in ponds or lakes are very low (below 5.3 μM) (Ndungu et al. 2019; Weenink et al. 2021). Reactive oxygen species, including H_2O_2, at a low concentration usually serve as important signaling molecules (Neill et al. 2002). Cyanobacteria are a diverse group of prokaryotic microorganisms that perform oxygenic photosynthesis and widely exist in freshwater, marine, and terrestrial ecosystems. The application of exogenous H_2O_2 in the treatment of toxic cyanobacteria is environmentally friendly because of its decomposition to O_2 and H_2O. The H_2O_2 dosage applied is crucial for the compositional changes in phytoplankton and zooplankton communities (Weenink et al. 2015, 2021). High doses of exogenous H_2O_2 (e.g., 0.06 ~ 0.29 mM) have been reported to selectively eliminate toxic cyanobacteria such as *Microcystis aeruginosa* and *Planktothrix agardhii*, with a minimal impact on eukaryotic phytoplankton and zooplankton (Matthijs et al. 2012; Spoof et al. 2020; Santos et al. 2021). A biocidal level of H_2O_2 is supposed to trigger intracellular reactive oxygen species and result in severe oxidative stress and subsequent cell death (Drábková et al. 2007; Foo et al. 2020).

Many cyanobacteria are known to be able to synthesize extracellular polysaccharides or exopolysaccharides (EPSs) (Philippis and Vincenzini 1998). Although the functions of microbial EPS vary with the species, it serves as one of the primary mechanisms for cell survival in extreme habitats and defense against toxins, heavy metals, and antagonists (Rossi and De Philippis 2016; Bhatnagar and Bhatnagar

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2019). Cyanobacterial EPS also exhibits antioxidant activity (Huang et al. 2017; Zhong et al. 2019; Yuan et al. 2023), and antioxidant activity is associated with multiple factors, such as molecular weight, monosaccharide composition, sulfate position and sulfate degree (Mohan and Thirupathi 2021). It has been reported that H_2O_2 levels above 0.27 mM are lethal to *M. aeruginosa* (Foo et al. 2020). In contrast, another study showed that *Microcystis* populations with a colony size below 25 μm collapsed under 0.15 mM H_2O_2, while a H_2O_2 level of 0.59 mM was needed to control larger *Microcystis* colonies (Liu et al. 2017a). The formation of cyanobacterial colonies is mainly facilitated by EPS (Limoli et al. 2015). The difference in the biocidal level of H_2O_2 may hint that it is crucial for cyanobacterial cells to have a high abundance of EPS to combat external H_2O_2 stress.

A previous study reported that the extracellular polymeric substance of *M. aeruginosa*, mainly composed of polysaccharides and proteins (approximately 3:1 ratio in weight), showed the capability to buffer H_2O_2 (Gao et al. 2015). To clarify the crucial role of the EPS itself in combating exogenous high-dose H_2O_2, we evaluated the H_2O_2 buffering or scavenging capability as well as the protective role of the EPSs from four nonbloom-forming cyanobacteria with different thicknesses of extracellular polymers. This study deepens our understanding of the crucial role of EPS in combating external oxidative stress and provides reference information for guiding better application of H_2O_2 in environmental management.

Materials and methods

Cyanobacterial strains and culture conditions

The aquatic-living culture of *Nostoc flagelliforme* was previously prepared from a terrestrial natural sample in our laboratory (Cui et al. 2017). Its natural sample, presenting a filamentous colony form, consists of a large number of trichomes encased by dense EPS. *Nostoc punctiforme* PCC 73102 (hereafter referred to as *N. punctiforme*) was obtained from Pia Lindberg (Department of Chemistry-Ångström, Uppsala University), which can also produce a large amount of EPS (Soule et al. 2016). The *N. flagelliforme* and *N. punctiforme* strains were statically cultured in Blue Green-11 (BG-11) medium at 25 °C under constant white LED light of 40 μmol photons m^{-2} s^{-1}. Two freshwater strains, *Nostoc* sp. PCC 7120 (hereafter referred to as *Nostoc* 7120) and *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis* 6803), were statically cultured in BG-11 medium at 30℃ under the same light intensity. These cultures were gently shaken three times per day. Cell cultures at the exponential growth phase, with an optical density at 730 nm (OD_{730}) of 0.2 ~ 0.3, were used for subsequent experiments.

Analysis of the H_2O_2 tolerance of four cyanobacteria

The H_2O_2 stock solution (30%, v/v; Damao®, Tianjin Damao Chemical Reagent Factory, China) was diluted to 100 mM with sterile water for use. Cell cultures of 20 mL were used to test the tolerance to exogenous H_2O_2 in 50 mL conical flasks. The cell cultures were first treated with 2 mM or 4 mM H_2O_2 (final concentration). For a rapid estimation of the physiological status of cells, the chlorophyll fluorescence parameter *Fv'/Fm'* was determined, which reflects the photochemical efficiency of open photosystem II centers under a given light acclimation status (Campbell et al. 1998). At different time points (0~24 h), 2 mL of cell culture was collected and subjected to detection using a portable Plant Efficiency Analyzer (AquaPen FP110, Czech Republic) according to the manufacturer's instructions.

For the cell growth assay, cell cultures of 20 mL were treated with various concentrations of H_2O_2 (0 ~ 20 mM, final concentration). Then, the H_2O_2-treated cell cultures were cultivated for 4 days under the abovementioned conditions. Cell growth was monitored by measuring the concentration of chlorophyll a (Chl a). Chl a was extracted and quantified as previously described (Zhao et al. 2008). Briefly, 3 mL of cell suspension was concentrated by centrifugation at 6000 rpm for 5 min (TGL-16 M, Hunan Xiangyi Laboratory Instrument Inc., China) and then extracted with 3 mL 95% ethanol overnight at 4 °C. The absorbance was measured at 664.1 and 648.6 nm using an ultraviolet−visible spectrophotometer (Shanghai Spectrum Instruments Inc., China). The concentration of Chl a was calculated using the following equation: Chl a ($\mu g\ mL^{-1}$) = $13.36 \times A_{664.1} - 5.19 \times A_{648.6}$ (Lichtenthaler and Buschmann 2001).

Extraction and purification of EPS from cell cultures

The extraction of EPS was conducted with hot water as previously described (Huang et al. 1998) with slight modification. In brief, 10 mL of cell culture was extracted in 95 °C hot water for 2 h, and after centrifugation, the polysaccharide in the supernatant was precipitated overnight at 4 °C with ethanol (final concentration 80%, v/v). The crude polysaccharide was collected, dissolved in distilled water, and deproteinized with Sevag reagent (n-butanol:chloroform = 1:4, v/v) (Zheng et al. 2021). The purified EPS was freeze-dried, weighed, and dissolved in distilled water for use. The polysaccharide content was determined using the phenol−sulfuric acid method (DuBois et al. 1956). To verify that there was no contamination of proteins, the polysaccharide solution (1 mg mL^{-1}) was scanned by an ultraviolet−visible spectrophotometer from 200 to 400 nm.

Analysis of the H_2O_2 scavenging ability of the EPSs

The purified polysaccharide was added to a 5 mL H_2O_2 solution (2 mM) with final concentrations of 1 and 5 mg mL^{-1}.

The H_2O_2 solution without the polysaccharide addition (0 mg mL^{-1}) was used for comparison. Then, the H_2O_2 concentration in the solution was determined at different time points (0~3 h) using the Hydrogen Peroxide Assay Kit (Beyotime Biotechnology Inc, Shanghai, China) according to the manufacturer's instructions (Zang et al. 2018). The kit uses H_2O_2 to oxidize Fe^{2+} to Fe^{3+}, leading to the formation of a purple color in a specific solution. Absorbance was read at 560 nm using a microplate reader (Infinite M Nano, Tecan, USA). The relative changes (%) in H_2O_2 concentration were calculated.

Analysis of EPS protection of cells against exogenous H_2O_2

The above purified polysaccharides from the *N. flagelliforme* and *N. punctiforme* cultures were added into the cell cultures (20 mL) of *Synechocystis* 6803 and *Nostoc* 7120, respectively, with a final concentration of 0.05 or 0.10 mg mL^{-1}. The control cultures were not supplemented with the polysaccharides (0 mg mL^{-1}). Then, the cultures were treated with 1 mM H_2O_2 (final concentration). Subsequently, the physiological response of the treated cells, in terms of the *Fv'/Fm'* value, was analyzed at different time points (0~6 h). In addition, cell growth was monitored by measuring the Chl a content at 0, 2, and 4 days.

Analysis of the H_2O_2 scavenging ability of cell lysates

The cell lysates of four cyanobacteria were prepared as previously described (Weenink et al. 2021). In brief, 5 mL of cell culture was centrifuged at 6000 rpm for 5 min, and the cell pellets were resuspended in 5 mL phosphate buffered solution (0.01 M, pH 7.4). The cells were further disrupted at 4 °C in a 10 mL glass homogenizer (tissue grinding tube) and were ground manually with a ground glass pestle in the tube for 10 min. The cell debris was removed by centrifugation at 4 °C for 5 min. The supernatant (cell lysate) was collected and subjected to protein detection by the Bradford method (Bradford 1976) and polysaccharide detection by the phenol−sulfuric acid method (DuBois et al. 1956). The ratio of the polysaccharide to protein (mg mg^{-1}) in cell lysates was calculated. Furthermore, the scavenging abilities of cell lysates against H_2O_2 were assayed in 5 mL H_2O_2 solution (1 mM) by supplementing the cell lysate containing 0.5 mg soluble proteins. The H_2O_2 concentration in the solution was measured, and the relative change (%) was calculated.

Statistical analysis

For *Fv'/Fm'* detection, six replicates were performed. Other experiments except the growth assay in response to a wide concentration range of H_2O_2 were performed in three replicates. For column diagrams, the statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test ($p < 0.05$) (IBM SPSS Statistics 26).

Results

Different tolerances of four cyanobacteria to exogenous H_2O_2

In this study, four cyanobacteria available in our laboratory were used for experiments. Three of them, *Synechocystis* 6803, *Nostoc* 7120, and *N. punctiforme*, are widely used model strains; the last strain, *N. flagelliforme*, is becoming a new model strain, particularly along with its genome being sequenced (Shang et al. 2019). *Synechocystis* 6803 and *Nostoc* 7120 strains with thin EPS envelopes are referred to here as EPS-thin strains; *N. punctiforme* and *N. flagelliforme* strains are usually present as visible particles or colonies in culture due to their abundant EPS and are referred to here as EPS-rich strains. The responses of their cells to exogenous H_2O_2 were first assessed in a short-term test (Fig. 1). *Fv'/Fm'* serves as a quick indicator of the photosynthetic performance of algae and plants under abiotic stresses (Singh and Raja Reddy 2011; She et al. 2022). In terms of *Fv'/Fm'*, the physiological activities of the two EPS-thin strains

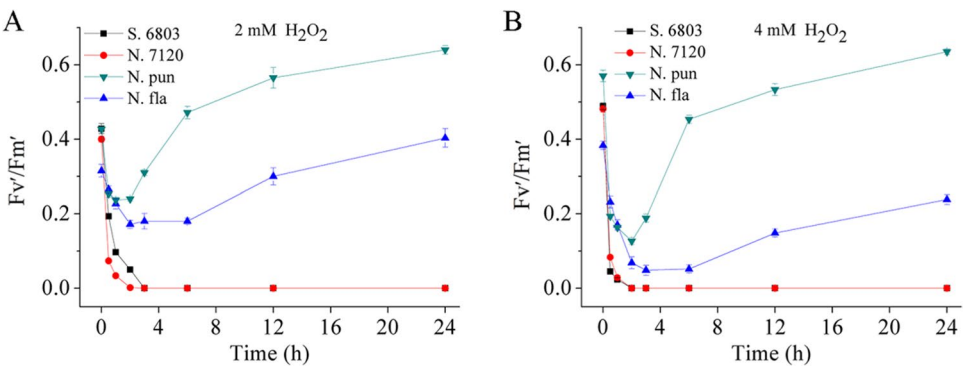

Fig. 1 Photophysiological activities (in terms of *Fv'/Fm'*) of four cyanobacteria in response to exogenous H_2O_2. **A**, the treatment of cultures with 2 mM H_2O_2. **B**, the treatment of cultures with 4 mM H_2O_2. Data shown are the mean ± SD ($n = 6$). S. 6803, *Synechocystis* sp. PCC 6803; N. 7120, *Nostoc* sp. PCC 7120; N. pun, *Nostoc punctiforme* PCC 73102; N. fla, *Nostoc flagelliforme*

were rapidly reduced to zero after either 2 or 4 mM H_2O_2 treatment. However, the physiological activities of the two EPS-rich strains were rapidly inhibited in the initial several hours and then gradually recovered; unlike the *N. punctiforme* strain, the activity of the *N. flagelliforme* strain did not recover to its original level under 4 mM H_2O_2 conditions.

Furthermore, the cell growth of the four strains, in terms of Chl a content, was assessed in response to exogenous H_2O_2 (Fig. 2). In the relatively long-term treatment (4 days), an expanded concentration range of H_2O_2 was used. The *Synechocystis* 6803 and *Nostoc* 7120 strains showed similar tolerance to H_2O_2, and both strains were not tolerant to 2 mM H_2O_2. In contrast, *N. punctiforme* and *N. flagelliforme* strains were much more tolerant to H_2O_2. The *N. punctiforme* strain was not tolerant to H_2O_2 levels above 16 mM, while the *N. flagelliforme* strain was not tolerant to H_2O_2 levels above 4 mM. The appearances of these cultures in glass flasks after H_2O_2 treatment were consistent with the above observations (Supplementary Fig. S1).

The scavenging ability of the EPSs against H_2O_2

The EPS encases the cyanobacterial cell and can also facilitate the formation of the colony; thus, its protective function by scavenging H_2O_2 should be important. The H_2O_2 scavenging abilities of the EPSs from four cyanobacteria were assayed (Fig. 3). The EPSs were extracted from the four cell cultures with proteins removed. A slow natural decomposition of H_2O_2 was observed, but all the EPSs could greatly accelerate the decomposition rate. At 3 h, 31.1% and 63.0% of the initial H_2O_2 was scavenged by 1 and 5 mg mL^{-1} *Synechocystis* 6803 EPS, respectively; the scavenging rates were 34.0% and 61.3% for 1 and 5 mg mL^{-1} *Nostoc* 7120 EPS, 43.8% and 72.1% for 1 and 5 mg mL^{-1} *N. punctiforme* EPS, and 35.0% and 70.7% for 1 and 5 mg mL^{-1} *N. flagelliforme* EPS, respectively. It was also noted that rapid H_2O_2 decomposition by these EPSs was observed within 1 h.

Protection of the EPSs against exogenous H_2O_2 in cyanobacteria

As shown in Figs. 1 and 2, *Synechocystis* 6803 and *Nostoc* 7120 were more sensitive to H_2O_2. Thus, their cultures were chosen for physiological activity and cell growth assays upon H_2O_2 treatment after supplementation with additional EPSs. The physiological activity (in terms of *Fv'/Fm'*) changes of *Synechocystis* 6803 and *Nostoc* 7120 cells against H_2O_2 were first assayed (Fig. 4). In the absence of additional EPSs (as the control), the physiological activities of the H_2O_2-treated *Synechocystis* 6803 and *Nostoc* 7120 cells rapidly decreased to zero or near zero within 3 h. In the presence of additional EPSs, the physiological activities of *Synechocystis* 6803 cells were stimulated within 1 h, slightly reduced at 3 h, and then recovered to a steady level at 6 h, while the physiological activities of *Nostoc* 7120 cells were rapidly reduced within

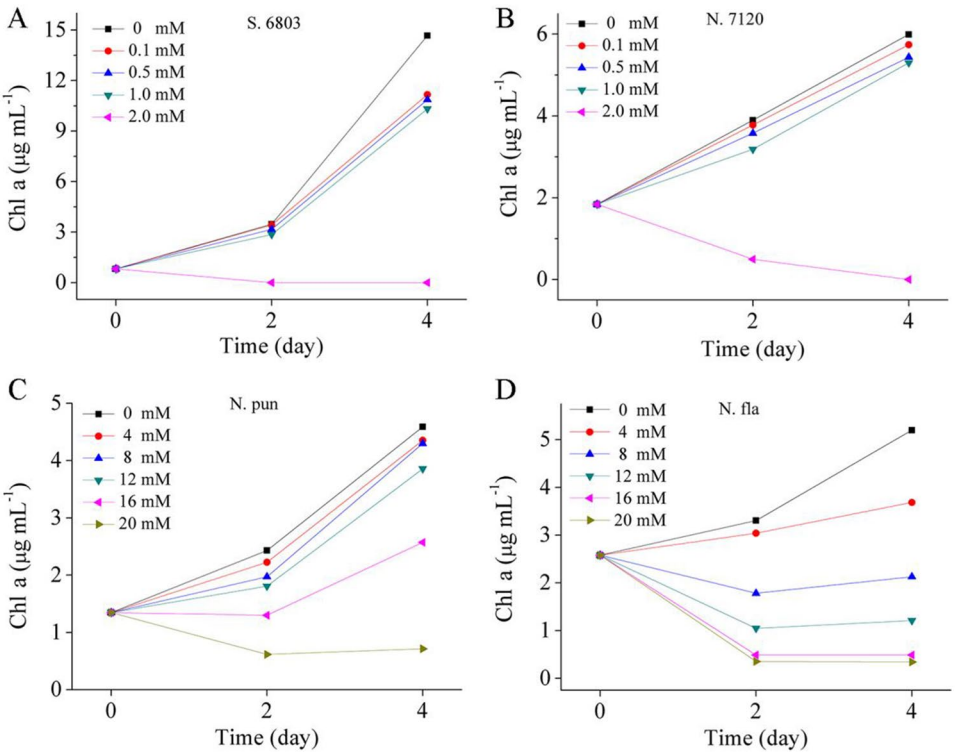

Fig. 2 The cell growth (in terms of Chl a content) of four cyanobacteria after treatment with exogenous H_2O_2. **A** and **B**, the growth of *Synechocystis* 6803 and *Nostoc* 7120, respectively. Cells were treated with 0 ~ 2.0 mM H_2O_2. **C** and **D**, the growth of *N. punctiforme* and *N. flagelliforme*, respectively. Cells were treated with 0 ~ 20 mM H_2O_2

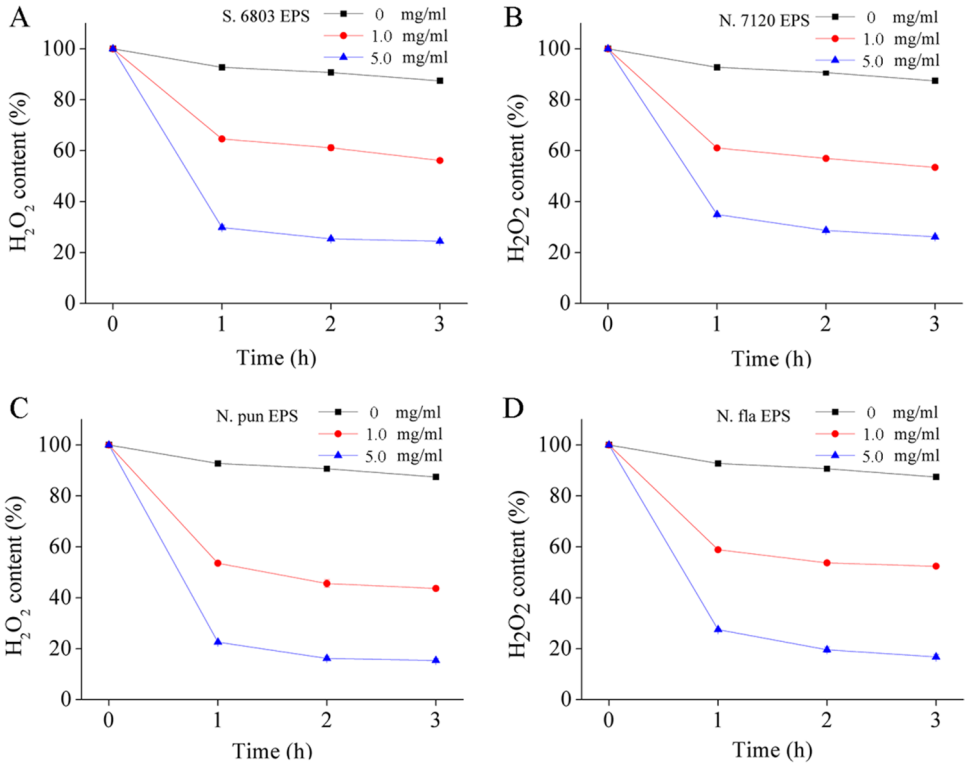

Fig. 3 The scavenging ability of four EPSs against H_2O_2. **A**, the *Synechocystis* 6803 EPS. **B**, the *Nostoc* 7120 EPS. **C**, the *N. punctiforme* EPS. **D**, the *N. flagelliforme* EPS. The polysaccharide concentrations in 2 mM H_2O_2 solution were 0, 1.0, and 5.0 mg $mL.^{-1}$. The relative changes (%) in H_2O_2 concentration in the solutions were calculated. Data shown are the mean ± SD ($n = 3$)

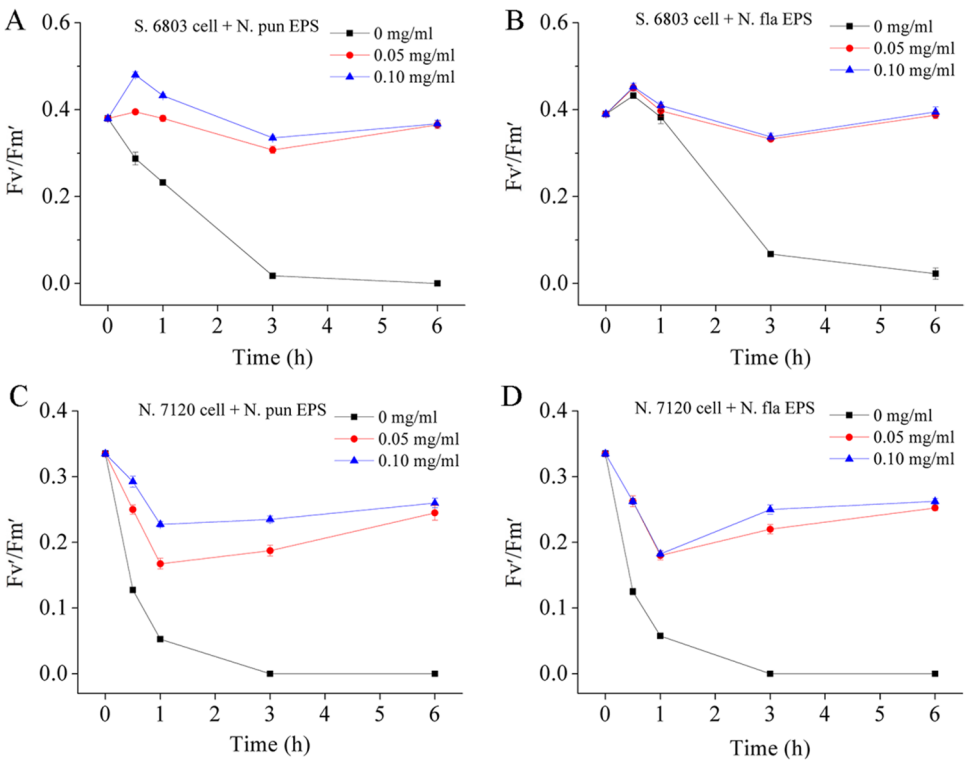

Fig. 4 Photophysiological activities (in terms of *Fv′/Fm′*) of two EPS-thin cyanobacteria after H_2O_2 treatment in the presence of additional EPSs. The concentrations of additional EPSs in the cultures were 0, 0.05, or 0.10 mg $mL.^{-1}$, and then the cultures were treated with 1.0 mM H_2O_2. **A** and **B**, *Synechocystis* 6803 cells protected by *N. punctiforme* EPS and *N. flagelliforme* EPS, respectively. **C** and **D**, *Nostoc* 7120 cells protected by *N. punctiforme* EPS and *N. flagelliforme* EPS, respectively. Data shown are the mean ± SD ($n = 6$)

1 h and then recovered to some extent at 3 or 6 h. Furthermore, the cell growth (in terms of Chl a content) of *Synechocystis* 6803 and *Nostoc* 7120 after H_2O_2 treatment was assayed (Fig. 5). Cells were cultivated for 4 days following H_2O_2 treatment. With supplementation of additional EPSs, both *Synechocystis* 6803 and *Nostoc* 7120 cells showed better growth than their corresponding controls without EPS supplementation.

The H_2O_2 scavenging ability of cell lysates from four cyanobacteria

When the H_2O_2 dosage exceeds the scavenging capacity of the total polysaccharide, protection from intracellular antioxidant substances should also be important. Here, we evaluated the H_2O_2 scavenging ability of cell lysates from four cyanobacteria (Fig. 6). The cell lysates were characterized by detecting the protein and polysaccharide contents. The content ratios of polysaccharides to proteins were evaluated (Fig. 6A). The EPS/protein ratios of *N. punctiforme* and *N. flagelliforme* cells were 2.6- and 3.0-fold higher than those (0.31) of *Synechocystis* 6803 and *Nostoc* 7120 cells, respectively. The H_2O_2 scavenging abilities of the cell lysates were further assayed (Fig. 6B). Overall, all the cell lysates could efficiently scavenge H_2O_2. Despite having a much higher EPS/protein ratio, the cell lysates of the *N. punctiforme* and *N. flagelliforme* strains only showed slightly higher scavenging activities (below 5%) than those of the *Synechocystis* 6803 and *Nostoc* 7120 strains.

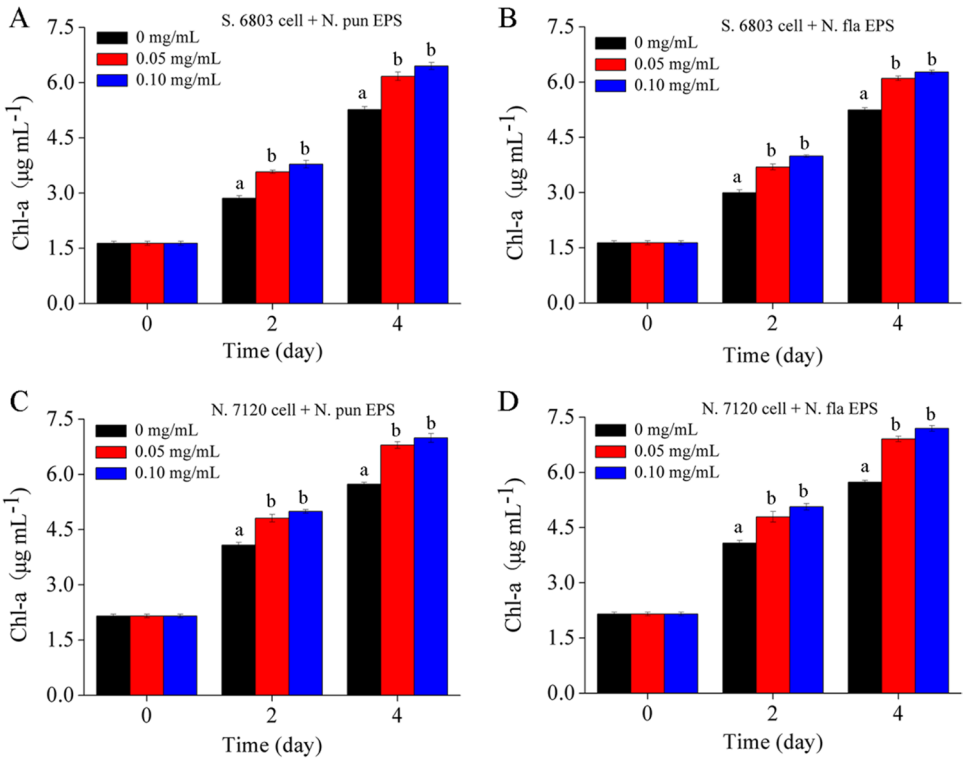

Fig. 5 The cell growth (in terms of Chl a content) of two EPS-thin cyanobacteria after H_2O_2 treatment in the presence of additional EPSs. The concentrations of additional EPSs in the cultures were 0, 0.05, or 0.10 mg $mL.^{-1}$, and the cultures were treated with 1.0 mM H_2O_2. **A** and **B**, *Synechocystis* 6803 cells protected by *N. punctiforme* EPS and *N. flagelliforme* EPS, respectively. **C** and **D**, *Nostoc* 7120 cells protected by *N. punctiforme* EPS and *N. flagelliforme* EPS, respectively. The letters (a, b) in each group of data suggest significant differences ($P < 0.05$, Tukey's test). Data shown are the mean ± SD ($n = 3$)

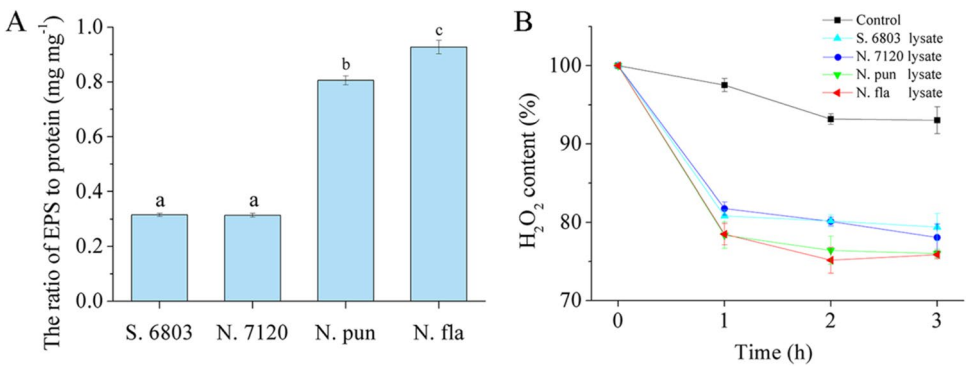

Fig. 6 The scavenging ability of cell lysates from four cyanobacteria against exogenous H_2O_2. **A**, the relative contents of EPS to protein in cell lysates. The letters (a, b, c) on the columns suggest significant differences ($P < 0.05$, Tukey's test). **B**, the H_2O_2 scavenging ability of cell lysates. Each cell lysate with an equal protein level (0.5 mg) was added to the H_2O_2 solution. The relative change (%) in H_2O_2 content in the solution was calculated. Data shown are the mean ± SD ($n = 3$)

Discussion

Although a biocidal level of H_2O_2 has been widely applied in controlling boom-forming cyanobacteria, the resilience of other cyanobacteria to cope with a high dose of exogenous H_2O_2 is not well known. Extracellular polymeric substances have been reported to be crucial for combating various environmental stresses (Gao et al. 2015, 2019; Tamaru et al. 2005; Liu et al. 2017b). In the extracellular polymeric substance of *M. aeruginosa*, the protein content varied in a large range, with the EPS/protein ratio being 0.24 ~ 2.88:1 (Gao et al. 2015; Ni et al. 2017). Both polysaccharides and proteins function in H_2O_2 decomposition (Gao et al. 2015). However, studies of terrestrial cyanobacteria have shown that extracellular polymeric substances contain only a minor proportion of exoproteins (Stuart et al. 2016; Shirkey et al. 2000). Aquatic toxic cyanobacteria seem to have a relatively larger amount of exoproteins in their extracellular polymers. Taking the four nonbloom-forming cyanobacteria with emphasis on the polysaccharide itself, we further clarified the importance of EPS in combating exogenous high-dose H_2O_2.

The EPS-rich cyanobacteria were much more tolerant to exogenous H_2O_2 than the EPS-thin cyanobacteria (Figs. 1 and 2). Further investigation showed that the EPSs of four cyanobacteria exhibited efficient H_2O_2 scavenging capability (Fig. 3) and seemed to provide rapid and nonselective protection for cells (Figs. 4 and 5). However, the physiological responses of *Synechocystis* 6803 and *Nostoc* 7120 were somewhat different (Fig. 4), implying a species/strain-dependent difference in combating H_2O_2 stress. The H_2O_2 decomposition rate was positively associated with the concentration of EPS (Fig. 3), implying that the polysaccharide amount plays a critical role. The H_2O_2 scavenging ability of the cell lysate was also observed (Fig. 6), which might help rescue the damaged cells to recover vitality. In previous studies, the activities of polysaccharides were mostly biochemically evaluated with the sensitive substrates 2,2-diphenyl-1-picrylhydrazyl, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid, and hydroxyl radical (Zhong et al. 2019). With *N. flagelliforme* as an example, cultural conditions and environmental treatment could also affect the antioxidant activity of the produced EPS (Shen et al. 2018; Yuan et al. 2023). In contrast, this study demonstrated a similar capability of four EPSs in combating a high dose of H_2O_2. Due to its phycosphere distribution, the amount of EPS should play a crucial role in cells against exogenous strong oxidative stress.

Although the cyanobacteria used in this study were not bloom-forming strains, they should still shed light on the understanding of environmental management. As implied in Fig. 3, the scavenging ability of the EPSs for exogenous H_2O_2 was rapid but also limited. In other words, the EPS amount plays a pivotal role. H_2O_2 sensitivity has been reported to sometimes be very different in the same strains (Liu et al. 2017a; Foo et al. 2020). The H_2O_2 tolerance concentrations of the *N. punctiforme* and *Nostoc* 7120 strains shown in this study were also different from those reported by Samanta et al. (Samanta et al. 2022). The reasons might be largely attributed to the EPS content per cell, colony size (still relevant to the EPS content), or ambient polysaccharide amount. In addition, the H_2O_2 sensitivity of cyanobacterial cells also depends on other factors, such as nutrient availability, metal ions, light intensity, heterotrophic bacteria, and eukaryotic algae (Drábková et al. 2007; Shen et al. 2011; Xu et al. 2016; Sandrini et al. 2020; Weenink et al. 2021; Moreno-Andrés et al. 2022), which increases the difficulty in precisely assessing the H_2O_2 dosage for environmental use. Considering the first-line role of EPS in H_2O_2 decomposition, the total polysaccharide amount in freshwater may be a primary consideration in determining H_2O_2 dosage for application.

In summary, this study suggests that cyanobacterial EPSs play a general role against exogenous high-dose H_2O_2 and also the polysaccharide amount plays a crucial role in protecting cells against external strong oxidative stress. This study provides a useful reference for the application of H_2O_2 in environmental management.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-023-26840-6.

Authors contributions Xiang Gao: Conceptualization, Supervision, Data curation, Writing-original draft and review & editing. Tao Zheng: Investigation, Methodology, Visualization, Writing-original draft. Xiaolong Yuan: Methodology, Investigation, Validation. Yibei Dong and Chang Liu: Investigation.

Funding This work has been supported by the Natural Science Basic Research Program of Shaanxi Province, China (No. 2020JZ-51) and the Shaanxi Province Qin Chuangyuan "Scientist + Engineer" Team Construction Project (No. 2023KXJ-206).

Data availability All data supporting the findings of this study are included in the main article and its supplementary files.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests The authors declare no conflict of interest.

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