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Effects of lanthanum on *Microcystis aeruginosa*: Attention to the changes in composition and content of cellular microcystins

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ABSTRACT

Algal blooms threaten human health and aquatic ecosystem through the production of microcystins (MCs) by toxic strains. The accumulation of rare earth elements (REEs) in water affects the growth and physiological activities of algae. However, whether or how REEs affect cellular microcystins (MCs) is largely unknown. In this study, the effects of lanthanum ion [La(III)], a type of REE, on the MCs in *Microcystis aeruginosa* were investigated, and the mechanism of the effect was analyzed using ecological stoichiometry. The different concentrations of La(III) were selected to correlate environmental pollution status. Low-dose La(III) (0.2, 2.0, and 4.0 μM) exposure increased the total content of MCs and the percentage contents of microcystin-YR (MC-YR) and microcystin-LW (MC-LW) and decreased the percentage content of microcystin-LR (MC-LR). High-dose La(III) (8.0, 20, 40, and 60 μM) exposure decreased the total content of the MCs, increased the percentage content of MC-LR, and decreased the percentage contents of MC-YR and MC-LW. The changes in the total MCs content were positively associated with the ratios of C:P and N:P in algal cells. The composition of MCs was dependent on the ratio of C:N in algal cells; for example, the percentage content of MC-LR decreased and the percentage content of MC-YR and MC-LW increased as the ratio of C:N in algal cells increased. In conclusion, La(III) could affect the content and composition of MCs via changes in the growth and chlorophyll-*a* content of *Microcystis aeruginosa*, and these effects depended on the ratios of C:P, N:P, and C:N in *Microcystis aeruginosa*. Such changes may influence the toxicity of *Microcystis* blooms. The results provides a new insight into the mechanism of REEs effects on algal toxins and provide references for evaluating environmental risks of REEs pollution in aquatic ecosystems.

1. Introduction

Rare earth elements (REEs) with unique physical and chemical properties have applications in many areas, including chemical, military, aerospace, agricultural, and other fields (Massari and Ruberti, 2013; Thomas et al., 2014). The global demand for REEs has grown from 30,000 tons in the 1980s to about 135,000 tons in 2015 (Sun et al., 2017). Therefore, a large quantity of REEs have been released into the water. It has been reported that concentrations of REEs in groundwater ranged from 3.35 to 34.17 μg/L in Sikhote-Alin Ridge, Primorye (Kharitonova et al., 2017). In Lake Baikal, Russia, REEs concentrations in water ranged from 0.02 to 2.16 μg/L (Sklyarova et al., 2017). The concentration of light REEs was as high as 3294 pM in Han River, South Korea (Song et al., 2017). The accumulation of REEs in water causes potential harm to aquatic organisms (Prokop et al., 2015). Unfortunately, compared to heavy metals and metalloids, little research has been conducted on the effects of REEs on aquatic organisms (Liang et al., 2014). Therefore, the need for more detailed information about the effects of REEs on aquatic organisms is urgent.

Among aquatic organisms, algal cells can accumulate REEs from lake or river water, and the total amount of REEs in algal cells can reach 1.3 mg/kg, which is 10–20 times higher than concentrations found in terrestrial plants (Yan et al., 1998). Studies have shown that REEs can have positive or negative effects on the physiological processes and

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growth of microalgae, depending on the concentration of REEs (Chen et al., 2014; Qian et al., 2003; Wang et al., 2011; Yang et al., 2016). Low concentrations of REEs were found to have positive effects on the growth rate, photosynthesis (Qian et al., 2003), chlorophyll content, and protein and soluble sugar content of algae (Chen et al., 2014). REEs also may influence the enzymes of antioxidant defense systems, such as superoxide dismutase, peroxidase, and catalase (Wang et al., 2011; Yang et al., 2016). However, high concentrations of REEs can destroy the ultrastructure of algal cells (Wang et al., 2011).

Microcystins (MCs) are the most common cellular toxins produced by cyanobacteria (blue-green algae) in eutrophic water (Gorham et al., 2017). A large amount of cellular MCs can be released by lysed cells during algal blooms and pose an enormous threat to aquatic ecosystem (Li et al., 2016a). In previous studies, the effects of REEs on algae or cyanobacteria was focused on the changes of growth, photosynthesis, enzymes, ultrastructure, and extracellular microcystin-LR (MC-LR) (Prokop et al., 2015; Wang et al., 2011; Yang et al., 2016), whether or how REEs affect cellular MCs in cyanobacteria is still unknown. *Microcystis aeruginosa*, a species of freshwater cyanobacteria, 3–7 μm in diameter, lack individual mucilage sheaths and is photosynthetic prokaryotes. *M. aeruginosa* is favored by warm temperatures, grow at water temperatures above 15 °C, and widely distributed in warm lakes and ponds in the world. *M. aeruginosa* can produce MCs and become most common toxic cyanobacteria in eutrophic fresh water. Cellular nutrient elements, such as C and N, mineral elements, growth, and chlorophyll content of *M. aeruginosa* have been shown to be important factors affecting the concentrations of MCs (Deblois and Juneau, 2010; Gouvea et al., 2008; Waal et al., 2014). Therefore, in this study, *Microcystis aeruginosa* (FACHB-1752) was selected as the experimental subject and cultivated in culture solution containing different doses of lanthanum (III) [La(III)], a type of REE, to investigate the effects of REEs on the MCs in *Microcystis aeruginosa*, and the mechanism was investigated using ecological stoichiometry analysis by determining the concentrations and ratios of nutrient elements, chlorophyll-*a* (Chl-*a*), and the growth of *M. aeruginosa*. The results may provide new insight into the mechanism of REEs effects on MCs, further understanding of the effects of REEs on algal cells, and can provide experimental evidence for assessing how harmful algae might respond and evolve to REEs contamination in the near future.

2. Materials and methods

2.1. Materials

Microcystis aeruginosa (FACHB-1752) was purchased from Wuhan Freshwater Algae Culture Collection at the Institute of Hydrology, Chinese Academy of Sciences (Wuhan, China) and cultured in BG11 medium (Olvera-Ramirez et al., 2000; Yang et al., 2016).

$LaCl_3$ (Sinopharm Chemical Reagent Company, Shanghai, China) was dissolved in distilled water to prepare a stock solution of 1.0 mol/L. The stock solution was kept at 4 °C in a sealed container before use.

Water was purified by a Milli-Q filtration system (Millipore, Bedford, MA, USA). Methanol and acetonitrile (ACN) were of high performance liquid chromatography (HPLC) grade (CNW Technologies, Shanghai, China). Microcystin-LR (MC-LR), microcystin-YR (MC-YR), microcystin-RR (MC-RR), microcystin-LA (MC-LA), microcystin-LY (MC-LY), microcystin-LW (MC-LW), and microcystin-LF (MC-LF) were provided by Alexis/Enzo Biochemicals Company (Lausen, Switzerland).

2.2. Algal culture and La(III) treatment

Based on the environmental pollution status of REEs and studies on the mechanism of action of La(III) on microalgae (Jin et al., 2009; Lin et al., 2003; Qian et al., 2003; Qinhai et al., 1997; Song et al., 2000), a variety of concentrations of La(III) were selected to treat *M. aeruginosa*. The *M. aeruginosa* cells were cultured in 1000 mL BG11 medium and mixed with 0 (control), 0.2, 2.0, 4.0, 8.0, 20.0, 40.0, and 60.0 μM of La (III). The cultures were adjusted to pH 8.0 with HCl or NaOH (Jin et al., 2009). The initial density of *M. aeruginosa* was 1.0×10^5 cells/mL. Experiments were performed in Erlenmeyer flasks (2000 mL) in triplicate under 30 ± 1.0 °C with illumination of 35 μmol photons/m^2/s with a 12 h light/12 h dark cycle. The flasks were shaken slightly four times every day to maintain homogeneity (Bi et al., 2016).

After three weeks, *M. aeruginosa* reached a steady state during which samples were taken. Prior to analysis, *M. aeruginosa* cells were extracted using centrifugation for 25 min at 6000 rpm (Lin et al., 2003; Yang et al., 2016). Algal cells were rinsed 3 times with deionized water and then centrifuged to remove residual culture medium.

2.3. Measurement of MCs

The cells were stored freeze-dried at −25 °C, freeze-thawed twice and extracted with 2.0 mL of 75% aqueous methanol in a bath sonicator for 15 min. After the ultrasonic bath treatment, the samples were sonicated further using ultrasonic disruptor (Sonifier 250A, BRANSON, USA) for 1 min (Spoof et al., 2004). The extracts were centrifuged at 6000 rpm for 10 min. The supernatant was analyzed using an ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometer (API4000$^+$, AB SCIEX, USA). Separation of the MCs was performed using an ACQUITY BEH130C_{18} column (1.7 μm, 100 mm × 2.1 mm; 40 °C). The mobile phase was acetonitrile-H_2O containing 0.1% formic acid, and the flow rate was at 0.40 mL/min. The optimal gradient was acetonitrile, 20% (0–1 min), 20–95% (1–2.5 min), 95% (2.5–3.5 min), 20–90% (3.5–3.6 min), and 20% (3.6–5 min) (Shen et al., 2017a). The precursor ions of MC-RR, MC-YR, MC-LR, MC-LA, MC-LY, MC-LW, and MC-LF were found at m/z 520.0, 523.4, 498.4, 911.5, 1002.5, 1002.5, and 986.5, respectively, in this experiment (Shen et al., 2017b). An m/z of 135 was used as the product ion in tandem mass spectrophotometry (MS/MS) for determination of MCs.

2.4. Determination of carbon (C), nitrogen (N), phosphorus (P), and metal element contents

The C content of *M. aeruginosa* was measured by a TOC analyzer (TOC-VCPH, Shimadzu, Japan) (Bell et al., 2013; Xing et al., 2015).

The N content of *M. aeruginosa* was determined by the alkaline potassium persulfate oxidation-UV spectrophotometric method (Sun et al., 2015; Zhu et al., 2011).

The algal cells were dried at 65 °C for 24 h. A microwave digestion procedure in a multiwave sample preparation system (Multiwave3000, Anton Paar, Austria) was applied to dried samples (Rodushkin et al., 1999). To each sample, 5 mL nitric acid and 0.5 mL hydrogen peroxide were added in a Teflon perfluoroalkoxy digestion vessel. Microwave digestion was performed at 600 W for 1 h. The vessel was removed from the oven and cooled to 30 °C. The digest was diluted 5-fold with purified water, centrifuged at 4000 rpm for 5 min, and potassium, magnesium, iron, copper, calcium, manganese, cobalt, zinc, lanthanum, and P contents were analyzed with an inductively coupled plasma-optical emission spectrometer (ICP-OES 720, Agilent, USA).

2.5. Measurement of chlorophyll-a content and cell densities

Chl-*a* in *M. aeruginosa* was extracted using acetone (90%, V/V) for 20 h in darkness at 4 °C. The extracts were centrifuged at 6000 rpm for 15 min. The absorbance of the supernatant was measured at 750, 664, 647, and 630 nm using a UV–vis spectrophotometer (UV-2450, Shimadzu, Japan) (Tien and Chen, 2012).

The cell densities of *M. aeruginosa* were measured using a hemocytometer with a microscope (Yang et al., 2016), results were the mean of three identical experiments.