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Effect of microcystins on root growth, oxidative response, and exudation of rice (*Oryza sativa*)

Qing Cao[a,b], Richard R. Rediske[c], Lei Yao[a,b], Liqiang Xie[a,*]

[a] State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, 73 East Beijing Road, Nanjing 210008, China

[b] University of Chinese Academy of Sciences, Beijing 100049, China

[c] Annis Water Resources Institute, Grand Valley State University, 740 West Shoreline Drive, Muskegon, MI 49441, USA

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ABSTRACT

A 30 days indoor hydroponic experiment was carried out to evaluate the effect of microcystins (MCs) on rice root morphology and exudation, as well as bioaccumulation of MCs in rice. MCs were bioaccumulated in rice with the greatest concentrations being observed in the leaves (113.68 μg g^{-1} Fresh weight (FW)) when exposed to 500 μg L^{-1} MCs. Root activity at 500 μg L^{-1} decreased 37%, compared to the control. MCs also induced disruption of the antioxidant system and lipid peroxidation in rice roots. Root growth was significantly inhibited by MCs. Root weight, length; surface area and volume were significantly decreased, as well as crown root number and lateral root number. After 30 days exposure to MCs, an increase was found in tartaric acid and malic acid while the other organic acids were not affected. Glycine, tyrosine, and glutamate were the only amino acids stimulated at MCs concentrations of 500 μg L^{-1}. Similarly, dissolved organic carbon (DOC) and carbohydrate at 50 and 500 μg L^{-1} treatments were significantly increased. The increase of DOC and carbohydrate in root exudates was due to rice root membrane permeability changes induced by MCs. Overall, this study indicated that MCs significantly inhibited rice root growth and affected root exudation.

1. Introduction

Microcystins (MCs) are frequently produced by some bloom-forming cyanobacteria, mainly *Microcystis*, *Anabaena, Nostoc* and *Oscillatoria*, in eutrophic fresh water lakes. Owing to MCs' potential carcinogenicity, they can negatively affect both public health and fundamental ecological processes (Rastogi et al., 2014; Zhao et al., 2016). More than 200 different structural analogues of MCs, with a range of molecular weights from 882 to 1116 Da, have been identified from cyanobacterial blooms and cultures (Zastepa et al., 2015; Spoof and Catherine, 2017). Microcystin-LR is found to be the most common and potent analogue, followed by microcystin-RR and microcystin-YR (Chen et al., 2016). With the increasing prevalence of these cyanobacterial blooms, more attention is being paid to impacts caused by MCs.

Cyanobacterial blooms have become more frequent in recent years in many large freshwater lakes and reservoirs in China, especially Lake Taihu (Hu et al., 2016). Consequently, it is suspected that farmland regions adjacent to Taihu lakes, which is one of the most intensive crop areas and densely populated areas in China, have become contaminated by irrigation water containing a cyanobacteria bloom. Rice crop take up about 75% of the arable land in this region (Zhang et al., 2003). Although MCs were produced in aquatic ecosystems, MCs can be brought into contact with land plants by irrigation source water that contains cyanobacteria; in addition, cyanobacterial blooms can be applied directly to soil as organic fertilizer after being intentionally harvested from lakes (Abe et al., 1996; Saqrane et al., 2009; Peuthert et al., 2007; Liu et al., 2008).

Several studies on effects of MCs on terrestrial plants showed that MCs can induce growth inhibition, accumulation of ROS and alteration of antioxidant systems (Chen et al., 2004, 2012; Gehringer et al., 2003; Yin et al., 2005; Lahrouni et al., 2012; Pichardo and Pflugmacher, 2011). However, there are hardly any studies focusing on the impact of MCs on plant root exudates, which play important roles in plant-soil system (Bais et al., 2006). In annual plant species, up to 70% of the photosynthetically fixed carbon can be transferred to the rhizosphere after being translocated to the roots (Lynch and Whipps, 1990; Liljeroth et al., 1994). Commonly, root exudates are classified into two categories, low-molecular weight compounds (organic acids, phenolic compounds, amino acids, carbohydrate and some secondary metabolites) and high-molecular-weight (mainly mucilage and proteins) (Vančura, 1988; Walker et al., 2003). The functions of most root exudates are still unclear due to their multicomponent composition,

* Corresponding author.
E-mail address: lqxie@niglas.ac.cn (L. Xie).

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whereas some of these compounds have been reported to enhance nutrient uptake (Jones et al., 1994), mobilization of soil nutrients (Subbarao et al., 1997) and weathering of soil minerals (Hinsinger and Jaillard, 1993). In addition, root exudates have been shown to be important in biodegradation of pollutants in the rhizosphere. It is reported that root exudates can improve pollutant bioavailability by modifying soil conditions (Ouvrard et al., 2006; Joner et al., 2002), promote the development and activity of soil microorganisms (Harvey et al., 2002; Yoshitomi and Shann, 2001), and transform/degrade the pollutants with root exuded organic acids (activation of abiotic oxidants) (Gramss, 2000) and root-driven extracellular enzymes (oxidation of pollutants) (Schnoor et al., 1995; Siciliano et al., 1998). In consideration of the use of MC contaminated watr for irrigation, it is important to explore the shifts in root exudates after MC exposure.

The main objective of this research was to explore the alterations in rice root after exposure to MCs, which includes MCs accumulation, root activity, biochemical response in root, root morphology, and root exudates. These findings help develop a better understanding of the impact of MCs on terrestrial plant communities.

2. Materials and methods

2.1. Extraction, purification and analysis of MCs variants

In the present study, we used a natural cyanobacterial bloom extract so as to mimic natural toxic conditions with multiple MCs. In order to prevent interference from impurities, the extract was purified with an Oasis HLB extraction cartridge (Waters). The method of extraction and purification was modified from Harada et al. (1988). Cyanobacterial cells obtained from Dianchi Lake (~ 1 g dry weight (DW)) were homogenized with 25 mL of 5% (v/v) aqueous acetic acid after freeze-drying. The homogenate was subjected to an ultrasonic bath for 5 min, and then centrifuged at 12,000 × *g* at 4 °C for 15 min. After re-extracting the residue two more times as before, all the supernatant was collected and then applied to 5 g HLB extraction cartridge. The cartridge with toxin was rinsed with 50 mL of 5% (v/v) aqueous methanol. Subsequently, the cartridge was eluted with 100 mL 100% methanol. The eluate was evaporated to dryness, and then 10 mL deionized water was used to dissolve the toxin. Combined toxin-containing solutions were stored at −40 °C before use. According to Corbel et al. (2015), MC variants were analyzed by ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC/MS), using a Waters Acquity UPLC system coupled to a triple-quadruple mass spectrometer (TQD, Waters, France) via an electrospray ionization (ESI) interface. The mobile phase was composed of 0.1% formic acid in water (A) and acetonitrile (B), and the linear gradient was as follows: 0–4.0 min, 75% A; 4.0–4.1 min, 55% A; 4.1–7.0 min, 75% A. The flow rate was 0.5 mL min^{-1} and the injected volume varied from 5 μL. The stationary phase was an Acquity UPLC BEH C_{18} column (100 mm × 1 mm, 1.7 μm). Some MS ion source parameters were: source temperature 150 °C, desolvation temperature 300 °C, cone gas flow 60 L h^{-1}, desolvation gas flow 640 L h^{-1}, capillary voltage 3 kV, positive ESI mode. MC-LR (14.92 mg L^{-1}), MC-YR (5.90 mg L^{-1}) and MC-RR (61.61 mg L^{-1}) were found in the extract after the analysis. The toxin extract was diluted as needed for subsequent use.

2.2. Plant culture and treatment

Seeds of rice (*Oryza sativa* L.) were sterilized with sodium hypochlorite (0.5%, w/v) for 30 min, followed by 3 washes with sterile distilled water and subsequently soaked with distilled water for 24 h at 37 °C in the dark. Then seeds were germinated for 7 days in trays containing sterile 10% strength Hoagland solution at 25 °C, at 300 μmol m^{-2} s^{-1} light intensity and a photoperiod of 12:12 L:D. After 7 days, plants were transferred to 2 L hydroponic cultures with either uncontaminated Hoagland solution (control), or solutions containing one of three concentrations of MCs (T5, T50 and T500, containing 5, 50 and 500 μg MCs, respectively). The black pots were covered by black PVC lids with holes (20 mm inner diameter) allowing the growth of rice plants on nylon nets. Each treatment included 4 replicates. Plants were cultivated in a growth chamber with a 14:10 L:D regimen (light intensity: 450 μmol m^{-2} s^{-1}; temperature: 30/28 °C; relative humidity: 70%) for 30 days. The nutrient solution was aerated and renewed every 3 days, and the MCs were re-added at the same time. No mortality was observed during the experiment.

2.3. Detection of MCs in plant tissues

The method of detection of MCs in plants was described by Saqrane et al. (2009) with slight modification. Briefly, about 2 g fresh plant tissue was ground with 2 mL of 70% aqueous methanol (v/v), and then homogenized with a total amount of 15 mL of 70% (v/v) aqueous methanol (v/v). The homogenate was subjected to an ultrasonic bath for 5 min, and then centrifuged at 10,000 r min^{-1} at 4 °C for 15 min. After re-extracting the residue two more times as before, all the supernatant was collected and evaporated to dryness with a rotary evaporator (EYELA N-1200B, Tokyo Rikakikai, Japan). Subsequently, 300 μL of 70% aqueous methanol (v/v) was used to dissolve the toxin. After being passed through a GF/C glass filter, the solution was subject to HPLC analysis.

2.4. Measurements of root biochemical indicator

Root activity was determined by the triphenyltetrazolium chloride (TTC) reduction method, specifically described by Li (2000).

Several test kits (purchased from NanJing JianCheng Bioengineering Institute, China) including Catalase (CAT) assay kit (Visible light), Total Superoxide Dismutase (T-SOD) assay kit (Hydroxylamine method), Total protein quantitative assay kit and Malondialdehyde (MDA) assay kit (TBA method) were used to measure CAT activity, total SOD activity, protein content and MDA content, according to the manufacturer's instructions.

2.5. Measurements of root morphology

Root morphological parameters (DW, length, surface area, volume, average diameter) were scanned with a root scanner (Perfection V700 Photo, Seiko Epson Corp, Japan), and analyzed by WinRHIZO 2009 software. Crown root number and lateral root number were also counted. After measurement of the root morphology, the roots were dried at 70 °C until constant DW, and weighed.

2.6. Collection of root exudates

Root exudates were collected according to Egle et al. (2003) after 30 days of cultivation. After lifting out of the beakers, rice roots were gently washed for two minutes to release rhizosphere solution. Subsequently, the rice plants were transferred to 250 mL beakers containing 200 mL 0.05 mmol L^{-1} $CaCl_2$ solution. To make a dark environment for the roots, tin foil was used to wrap the beakers. Then the plants were placed in the growth chamber under the same condition with plant cultivation. To get rid of cell contaminants, the $CaCl_2$ solution was renewed after 30 min, and Micropur®, an antimicrobial substance, was added. Root exudates were collected after 4 h cultivation under the same conditions. Right after collection, the root exudate solution was lyophilized and kept at −20 °C until analysis.

2.7. Analysis of root exudation

The root exudates were passed through a cation-exchange column (5 g of Amberlite IR-120B resin, 16 × 14 mm) (H^+ form, Muromachi Chemical, Tokyo, Japan), followed by an anion-exchange column (2 g