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Changes in metabolites, antioxidant system, and gene expression in *Microcystis aeruginosa* under sodium chloride stress

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ABSTRACT

Microcystis (*M.*) *aeruginosa*, one of the most common bloom-forming cyanobacteria, occurs worldwide. The Qingcaosha (QCS) Reservoir is undergoing eutrophication and faces the problem of saltwater intrusion. The aim of this study was to investigate the effects of sudden salinity changes on physiological parameters and related gene transcription in *M. aeruginosa* under controlled laboratory conditions. The results showed that sodium chloride (50, 200 and 500 mg L^{-1} NaCl) inhibited the algal growth and decreased pigment concentrations (chlorophyll *a*, carotenoid and phycocyanin). Sodium chloride increased both the intracellular and extracellular microcystin contents and elevated the *mcyD* transcript level in *M. aeruginosa*. It also increased the malondialdehyde (MDA) content and caused cytomembrane damage. This damage caused the release of intracellular toxins into the culture medium. In addition, NaCl decreased the maximum electron transport rate, increased the levels of reactive oxygen species (ROS) and changed the cellular redox status. Consequently, NaCl inhibited the expression of *cpcB*, *psbA* and *rbcL*. Furthermore, NaCl increased the activities of superoxide dismutases (SOD), catalase (CAT), glutathione reductase (GR), and total glutathione peroxidase (GPx). The transcript levels of *sod* and reduced glutathione (*gsh*) were also increased after exposure to NaCl. Our results indicate that a sudden increase in salinity increases the production and excretion of microcystin, changes the cellular redox status, enhances the activities of antioxidant enzymes, inhibits photosynthesis, and affects transcript levels of related genes in *M. aeruginosa*.

1. Introduction

Qingcaosha (QCS) Reservoir experiences saltwater intrusion and eutrophication. It is located in the Yangtze River estuary in China (Fig. 1), supplying drinking water to 11 million residents in Shanghai. Saltwater intrusion results in a relatively high chloride concentration in the reservoir. A previous study showed that chloride concentration in QCS Reservoir ranged between 12.21 and 216.07 mg L^{-1} (Jin et al., 2013). An analysis conducted in February 2014 revealed a chloride concentration as high as 3000 mg L^{-1}. Chloride concentration exceeding 250 mg L^{-1} can lead to an undesirable taste and may be hazardous to human health (McCarty, 2004). QCS Reservoir is undergoing eutrophication due to the relatively high nitrogen and phosphorus concentrations (1.62 and 0.092 mg L^{-1}, respectively) (Jin et al., 2013). In addition, QCS Reservoir is shallow, making it more vulnerable to algal blooms. Algal blooms increase the turbidity and lead to the deterioration of water quality, negatively affecting the growth of aquatic organisms and destroying the aquatic ecosystems' balance (Paerl and Huisman, 2009; Scheffer, 2004). Blooms have also been observed in brackish-water environments from locations as far as San Francisco estuary in the USA, the Marina Reservoir in Singapore, the Swan River estuary in Australia and from the Baltic Sea in Europe (Gin et al., 2011; Lehman et al., 2010; Lopes and Vasconcelos, 2011; Orr et al., 2004).

Saltwater intrusion has been found to change the environment for algal growth. High chloride concentration can result in the release of high levels of inorganic phosphorus from sediments (Jin et al., 2013; Williams et al., 2014). In addition, saltwater intrusion can change nitrogen retention and cycling in coastal freshwater wetlands, leading to increased nitrogen loading to sensitive coastal waters (Ardón et al., 2013). Consequently, these may cause potential development of toxic algal blooms. This is because phosphorus and nitrogen play important roles in algal growth.

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Moreover, salinity affects N_2 fixation in *Anabaena aphanizomenoides* and *cylindrospermopis*, which are isolated from estuary and freshwater systems (Moisander et al., 2002). It has also been found that saltwater intrusion increases water depth and consequently reduces available light to the bottom (Short and Neckles, 1999).

Microcystis aeruginosa (*M. aeruginosa*), a common bloom-forming cyanobacterium, can float on the water surface. In addition, it produces powerful toxins called microcystins (MCs) that can destroy the liver structure, cause health hazards to humans and animals, and even cause death in acute cases (Isaacs et al., 2014; Mattos et al., 2014; Qian et al., 2010). *M. aeruginosa* can survive after exposure to a series of salinities, but the salinity stress causes the leakage of cells and the excretion of the MC (Tonk et al., 2007). The toxicity levels between mature and developing blooms have been studied. Blackburn et al. (1996) reported that mature *Nodularia spumigena* blooms were more toxic than developing ones under salinity stress. Harmful algal blooms in brackish-water occur worldwide. Therefore, it is vital to focus on the effect of saltwater intrusion on algae growth and the subsequent toxicity.

Field studies on the growth and toxin production in *M. aeruginosa* under salinity stress have been carried out previously. In one study, MC production by Kucukcekmece lagoon strains was influenced by environmental factors such as nutrients and salinity (Albay et al., 2005). Similarly, lower salinities in San Francisco Bay Estuary lead to comparatively high concentration of MCs (Lehman et al., 2005). In addition, some laboratory studies have demonstrated that sodium chloride affected the production of metabolites and photosynthesis in cyanobacteria (Black et al., 2011; Hu et al., 2014; Lu and Vonshak, 2002; Zhang et al., 2013). Most of these studies only measure the changes of physiological parameters after exposure to sodium chloride, whereas information on the transcriptional regulation of related genes in *M. aeruginosa* is still scarce. Moreover, the possible mechanism through which salinity influences *M. aeruginosa* still remains unknown. The aim of this work was to study the influence of saltwater intrusion on gene transcription and its mechanism. The influence of salinity on growth, MC concentration, pigment content, the maximum electron transport rate, ROS level, MDA content, activity of antioxidant enzyme and gene expression was also investigated. This study can contribute profound understanding of the relationship between saltwater intrusion and harmful algal blooms in QCS Reservoir.

2. Materials and methods

2.1. Strain and culture conditions

M. aeruginosa (code: FACHB-911) was obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Batch cultures of *M. aeruginosa* were grown in BG-11 medium, maintained at 25 ± 1 °C under a 12/12 h light/dark cycle and illuminated with cool white fluorescent tubes (2000 lx). The growth of the cultures (control and treatment) was monitored by counting cell numbers and detecting optical density. Algal cultures were cultivated in 0, 50, 200, 500 mg L^{-1} sodium chloride (NaCl) for 12 days. All samples were prepared in triplicate. The initial *M. aeruginosa* density was 3.8×10^5 cell mL^{-1}.

2.2. Pigment content and maximum electron transport rate

The chlorophyll *a* (chl-*a*) concentration and maximum electron transport rate (ETRmax) were determined at room temperature using a PHYTO-PAM phytoplankton analyzer (Water-PAM, Walz, Germany). This was performed according to Gera's method (Gera et al., 2012). Cells were kept in the dark for 7.5 min before measurement.

Carotenoid examination involved extracting cells in 80% (v/v) acetone (Arnon, 1949). The samples were thrice frozen in liquid nitrogen and thawed at 4 °C. The samples were then kept in the dark for 24 h at 4 °C. The samples were later centrifuged for 10 min at 10,000 g and 4 °C. The carotenoid concentration was then calculated using the Carl method (Carl and Terence, 1998).

Phycocyanin (PC) is a water soluble pigment which was measured according to the Bennett method (Allen and Lawrence, 1973; Seyedeh and Nik, 2013).

2.3. Toxin analysis

The harvested cells were centrifuged at 10,000 g for 10 min at 4 °C. The supernatants were kept for extracellular MC detection. The pellets were suspended in PBS solution. The samples were thrice frozen in liquid nitrogen and thawed at 4 °C in order to rupture the cells. The ruptured cells were centrifuged at 10,000 g for 10 min at 4 °C, to obtain the solution containing intracellular MC. All supernatants were diluted with Milli-Q water and detected with an enzyme linked immunosorbent assay (ELISA) method. ELISA was performed using the QuantiPlate™ Kit for Microcystin (EnviroLogix, USA) according to the manufacturer's instructions.

2.4. Analysis of ROS level and lipid peroxidation

The effect of NaCl on ROS level was investigated by using the cell permeable indicator 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Beyotime Institute of Biotechnology, China). The fluorescence was measured at an excitation wavelength of 488 nm and emission wavelength of 525 nm using a Varioskan Flash spectral scanning multimode reader (Thermo Fisher Scientific, USA).

Lipid peroxidation analysis was detected by estimating malondialdehyde (MDA) levels, using the thiobarbituric acid-reactive substances (TBARS) method. The MDA content was measured using the MDA detection kit (Beyotime Institute of Biotechnology, China) according to the manufacturer's instructions. The absorbance was measured at 532 nm using a Varioskan Flash spectral scanning multimode reader (Thermo Fisher Scientific, USA).

2.5. Antioxidant enzyme activity analysis

The activities of superoxide dismutases (SOD), glutathione reductase (GR) and total glutathione peroxidase (GPx) were detected in order to evaluate oxidative damage.

SOD activity was measured using a Total Superoxide Dismutase Assay Kit with WST-1 (Beyotime Institute of Biotechnology, China). One unit (U) of SOD activity was defined as the amount of enzyme inhibiting 50% of WST-1 photoreduction.

The activities of GR and GPx were measured according to the manufacturer's instructions using the Glutathione Reductase Kit and Total Glutathione Peroxidase Kit respectively (Beyotime Institute of Biotechnology, China).

The activities of these enzymes were expressed on a basis of protein content. Total protein content of the supernatant was determined using an enhanced BCA Protein Assay Kit (Beyotime Institute of Biotechnology, China).

2.6. RNA extraction, reverse transcription, and real-time PCR

Harvested cells were centrifuged at 10,000 g for 10 min at 4 °C. The pellets were stored at −80 °C until RNA extraction. Total RNA was extracted using the TaKaRa MiniBEST Universal RNA Extraction Kit (Takara, Dalian, China) according to the manufacture's protocol. The concentration and purity of RNA were determined by spectrophotometry (Nanodrop 2000, Thermo Fisher) at 260 nm