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Comprehensive assessment of three typical antibiotics on cyanobacteria (*Microcystis aeruginosa*): The impact and recovery capability

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ABSTRACT

This innovative study provided a comprehensive evaluation of the effects of three typical antibiotics exposures (cefradine, norfloxacin and amoxicillin) on *Microcystis aeruginosa* in two periods (exposure and post-exposure) at a new perspective. The results indicated that the irreversible growth inhibition of *M. aeruginosa* attributed to the norfloxacin in the exposure and the re-exposure stages. In contrast, although the algal cell size recovered to the control level after the exposure of 20 mg/L of cefradine, the significant stimulation on glutathione (GSH) still persisted even if the contaminants were removed. On the other hand, amoxicillin inhibited the activities of superoxide dismutase (SOD), GSH contents and the algal cell size in the exposure period while malonaldehyde (MDA) contents increased significantly in two periods.

1. Introduction

Antibiotics are extensively used in human and veterinary medicine to prevent or treat infectious diseases, as well as in aquaculture operations, depending on their inhibitory capacity for microorganisms (Kummerer, 2009). Due to the improper disposal by the current technology, antibiotic residues became one of the highly persistent pollutants and have also been found in natural waters (Gao et al., 2012; Tong et al., 2014). In particular, antibiotics are found in agriculture and in cattle production and these activities are important sources of high concentration level of contamination in soils and freshwaters (M. Miyata et al., 2011). At the same time, antibiotic residues affect the biomass and activity of microbes, induce occurrence of resistance genes and severely threaten the function of aquatic ecosystem in the natural waters (Amador et al., 2015; Li et al., 2016; Mohanta and Goel, 2014). Previous studies have demonstrated that antibiotics had significantly toxic effects on algal growth capacity and *chlorophyll a* content, especially for cyanobacteria, which are more vulnerable than other algal species in the given scope of exposure concentration, because of the cellular structure similar to bacteria (Chen and Guo, 2012; Crane et al., 2006; Liu et al., 2012b).

As the prevalent bloom-forming algae, *Microcystis aeruginosa* is widely present in most water bodies. The growth of cyanobacteria is generally regulated by various environmental factors, such as temperature, trace metals, the content of nitrogen (N), phosphorous (P) (Davis et al., 2009; Jiang et al., 2008). Recent studies found that some exogenous contaminants, which including endocrine disrupters, nonylphenol, heavy metals and similar, may also impact on the cyanobacterial growth (Perron and Juneau, 2011; Wang et al., 2007; Zeng et al., 2009). Previous studies showed the effects of antibiotic on *M. aeruginosa*, including the changes of algal growth capacity, photosynthetic efficiency and microcystin-production (Graham, 2010; Liu et al., 2016; Shang et al., 2015). At the same time, during the exposure procedure, the exogenous contaminants could induce oxidative stress and cause antioxidant responses for algae cell to adapt to the changes of the external environment, which could be involved in the regulation of algal growth and other biological processes (Krieger-Liszkay, 2001; Mallick, 2000). A few researchers focused on the change of antioxidant responses in microalgae cell, as an industrial contaminant, nonylphenol was found to stimulate the antioxidant responses in *C. vulgaris* and *Selenanstrum capricornutum* after a 24 h exposure, including glutathione (GSH), catalase (CAT) and peroxidase (POD) activities (Gao and Tam, 2011). At the same time, ampicillin and streptomycin were also reported to increase the activities of antioxidant enzymes system in *M. aeruginosa*, which were particularly associated with the effects on both growth capacity and microcystin-release (Qian et al., 2012a, 2012b). Moreover, there is an apparent correlation between antioxidant responses of algae and the removal of target contaminants, and GSH conjugation was thought to be the principal mechanism related to the degradation of chlorphenol by *Skeletonema costatum* (Shao Yang, 2002).

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The above studies showed that antioxidant responses may be a significant indicator to evaluate the effect of antibiotics on *M. aeruginosa* and the recovery of growth in algae.

In traditional toxicological studies, the test organism was exposed to the target compound for a given time and/or given range of concentrations, thus frequently failed to consider the performance of the organism after the exposure ended. For green algae, there are several reports suggesting that *Chlorella vulgaris* showed a rapid recovery capability in the photosynthetic activity from the nonylphenol-induced damage (Gao and Tam, 2011). Exposure to atrazine could result in an immediate, but reversible, inhibition of photosynthesis and growth capacity of *Pseudokirchneriella subcapitata* (Brain et al., 2012). A recovery of inhibition from the contaminant exposure was detected, which indicated that, as as long as possible post-exposure period was pivotal to obtain a recovery (Agostinho et al., 2012; Mcwilliam and Baird, 2002). Our previous study also indicated that the filtration and ingestion rate of the rotifers recovered after the exposure to imatinib (Yan et al., 2017). However, the recovery of *M. aeruginosa* after antibiotics exposure was still uninvestigated. Additionally, previous studies usually examined the impact on the test organism from different angles, such as growth capacity, physiological index or antioxidant responses. Due to the varied concentration set or species strain, it was really hard to obtain an overall comprehensive evaluation of a target compound on the given test organism, among different studies.

Thus, the present work aimed to gain insights into the impact of three typical antibiotics, cefradine, norfloxacin and amoxicillin on cyanobacteria (*Microcystis aeruginosa*). The algal growth capacity, the content of exopolysaccharides (EPS), photosynthetic activity and cell size changes, as well as superoxide dismutase (SOD) activity, the content of malonaldehyde (MDA) and GSH in *M. aeruginosa* after a 24 h exposure and a 24 h post-exposure were observed, respectively. We used principal component analysis (PCA) to evaluate whether and/or the impact of the three antibiotics and the recovery capability was compound-dependent.

2. Materials and methods

2.1. Mass culture of M. aeruginosa

M. aeruginosa (FACHB-905) was purchased from Freshwater Algae Culture Collection in Institute of Hydrobiology, Chinese Academy of Sciences. Under aseptic conditions, *M. aeruginosa* was inoculated in BG-11 medium that contains are shown as table S1, S2 in support material during the algae growth period. All algae were cultivated in a light intelligent incubator at 25 ± 1∘C under 4000 lx illumination on the photoperiod 12:12 (L: D). The conical flasks were shaken two times a day and we randomly replaced the position to avoid the precipitation.

2.2. Experimental design

2.2.1. Exposure test

M. aeruginosa were cultured up to the logarithmic growth phase and inoculated into a certain amount of BG-11 medium at an initial cell density of about 10×10^6 cells/mL, which were measured by an ultraviolet spectrophotometer (Wan et al., 2014; Yan et al., 2017). The test of *M. aeruginosa* was divided into nine groups with three parallel tests, in which three antibiotics (cefradine, amoxicillin and norfloxacin) were dissolved in 300 mL of *M. aeruginosa*, separately. The control has no antibiotic. The concentrations of cefradine were 1, 10 and 20 mg/L, respectively, and the concentrations design for amoxicillin and norfloxacin was all the same as cefradine. The entire experiment was carried on at 25 ± 1°C with a light–dark interval of 12:12 h (4000 lx). The algal density was counted at 0.00, 3.00, 6.00, 12.0 and 24.0 h, respectively. Meanwhile, the samples were collected after the 24 h exposure treatment to analyze other physiological and biochemical indexes, including the antioxidant enzyme activity, the content of extracellular polysaccharide and *chlorophyll a*, algal growth capacity and cell size changes.

2.2.2. Post-exposure test

After 24 h antibiotic stress, the algae were centrifuged at 4000 rpm and cultured in fresh BG-11 medium, and the initial algal density of post-exposure was set in accordance with the exposure test by adjusting the amount of medium. The temperature and light conditions of the post-exposure test were the same as above, and the corresponding indexes were determined during the 24 h post-exposure treatment.

2.3. Analytical methods

2.3.1. Chemical and analytical method

The antibiotics amoxicillin (> 98% purity), norfloxacin (> 98% purity) and cefradine (> 98% purity) used in this study were purchased from Yabang investment holding group CO., LTD. The particle size of algae was determined using a laser diffraction particle size analyzer (Shimadzu, SALD-2201). To study the change of physiological and biochemical characteristics of algae after the exposure and post-exposure, the content of soluble protein and activity assays of three enzymes were detected by coomassie brilliant blue assay kit, superoxide dismutase (SOD) assay kit, malonaldehyde (MDA) assay kit and glutathione (GSH) assay kit, respectively. All the assay kits were from Jiancheng Bioengineering Institute, Nanjing, China. The algal cells were isolated by centrifugation at 4°C for 15 min (12,000 rpm), then 10 mL of 100 mM phosphate buffer (pH =7.4) was immediately added to each tube. The homogenate was extracted by ultrasonic cell disruptor (Ningbo Xinzhi biological Polytron Technologies Co., Ltd., JY92-IIN), and centrifuged at 12,000 rpm for 15 min. The supernatant could be used to measure above the index (Janknegt et al., 2009).

2.3.2. The content of chlorophyll a

The suspension samples of *M. aeruginosa* (10 mL) were centrifuged at 4000 rpm for 10 min, and the supernatant was discarded. Then, the algal cells were resuspended in 90% acetone at 4°C, centrifuged at 4000 rpm for 10 min. The absorbance of supernatant was measured at 630, 645 and 663 nm, and the content of *chlorophyll a* in cultures of *M. aeruginosa* was determined (Kosakowska et al., 2007).

2.3.3. The content of extracellular polysaccharide

10 mL samples were centrifuged at 12,000 rpm at 4 °C for 20 min, and the supernatant was filtered through a filter membrane (WhatmanGF/C). The filtrate was transferred to a dialysis bag (MW: 3500) and dialyze for 72 h in deionized water, then the content of polysaccharide was determined by anthrone-sulfuric acid method.

2.3.4. Data analysis

All the figures were produced using Sigmaplot version 12.5 in this study. The statistical analysis was applied to identify the significant differences among treatments by analysis of variance (ANOVA) using SPSS 21.0. If the result was shown $p < 0.05$, there was a significant difference between two sets of data. And the comprehensive toxic evaluations of antibiotics on *M. aeruginosa* were achieved by PCA using SPSS 21.0 (Guillén-Casla et al., 2011; Shin et al., 2010; Liu et al., 2017).

3. Results and discussion

3.1. The algal response in the exposure process

3.1.1. The growth capacity and the algal cell size

During the 24 h exposure, it was found (Fig. 1) that whole test concentration of cefradine and 10, 20 mg/L of amoxicillin all showed an inhibition effect on the growth of *M. aeruginosa*, and the inhibition levels were positively correlated with antibiotics concentration. But the algae still kept a satisfactory growth capacity in the whole process, and