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Effects and Mechanisms of Cadmium on the Degradation of Microcystin-LR by *Sphingopyxis* sp. YF1

Fei Yang[1, 2], Yuqing Gu[1], Jieqiu Yang[1], Bo Huang[1], Tangjian Peng[1]*

1. Hunan Province Key Laboratory of Typical Environmental Pollution and Health Hazards, School of Public Health, Hengyang Medical School, University of South China, Hengyang 421001, China;

2. Hunan Provincial Key Laboratory of Clinical Epidemiology, Xiangya School of Public Health, Central South University, Changsha, Hunan 410078, China;

Abstract: In this study, we investigated the effects of cadmium concentration on the degradation of microcystin-LR by *Sphingopyxis* sp. YF1, a highly efficient microcystins degrading bacterium. Our results demonstrate that 0. 25 mg/L cadmium decreased the degradation rate at 30 min, but had no significant effect on the biodegradation of MC-LR after 60 min; however, 4 mg/L cadmium significantly reduced the biodegradation rate of MC-LR throught the time points tested. The morphologies of cells in the presence of 0. 25 mg/L cadmium and in the absence of cadmium were similar. However, aggregation of cells was observed at a cadmium concentration of 4 mg/L. At the concentrations of 0. 25 mg/L (for 30 min and 60 min) and 4 mg/L (for 60 min and 90 min), there was an increase in cell membrane permeability, along with a significant rise in intracellular ATP content within 30 minutes. However, no significant changes were observed in MDA content or in the expression of degradation genes. When treated with 4 mg/L cadmium, cell membrane permeability increased, while intracellular ATP content rose within 30 minutes but declined at 60 minutes. Furthermore, MDA content increased significantly, indicating that 4 mg/L cadmium could induce oxidative damage to cells. Additionally, the expression of the gene encoding the ATP synthase B subunit was upregulated. Cadmium exerted a substantial influence on the degradation rate, cell morphology, energy metabolism and oxidative stress state of *Sphingopyxis* sp. YF1 during microcystin-LR degradation process providing valuable insights into how heavy metal pollution impacts microbial-mediated microcystin-LR degradation. The present study demonstrated distinct effects of low and high concentrations of cadmium on the degradation of MC-LR by bacteria. Specifically, the high concentration of cadmium primarily impacts the biodegradation of MC-LR by inducing cellular damage through oxidative stress, rather than suppressing the expression of genes associated with MC-LR degradation.

Keywords: Cadmium; *Sphingopyxis* sp. YF1; MC-LR; Biodegradation; Mechanism

1. Introduction

A significant amount of nitrogen, phosphorus discharged to water bodies can cause substantial eutrophication due to the haphazard discharge of industrial and agricultural effluent, which leads to the outbreak of cyanobacterial blooms (Song 2016). *Microcystis* blooms are the most prevalent type of cyanobacterial blooms, particularly in China's three main eutrophic water systems - Dianchi, Taihu Lake, and Chaohu Lake. Bloom-forming cyanobacteria are capable of producing toxic secondary metabolites such as microcystins (MCs), of which MC-LR is one of the most frequently detected toxins (Zurawell et al. 2005; Yang et al. 2018a; Yang et al. 2018b; Wen et al. 2018; Wu et al. 2020). MCs can enter the human body through drinking polluted water, eating aquatic plants or animals that have been contaminated, or contact with human bodies through domestic and recreational used water. MCs are causative agents for metabolism disorders and can cause damages to multiple organs, and even result in cancer (Zurawell et al. 2005; Massey et al. 2018; Chorus et al. 2000). How to effectively and safely remove MCs has become a pressing issue as it has posed a severe threat to public health and the sustainable development of human civilization in the worldwide (Xie 2015).

MC-LR is very stable and resistant to high and low temperatures and pH fluctuations (Rastogi et al. 2014; Tsuji et al. 1994). MC-LR can be degraded by some native bacteria in natural environments. These bacteria can change the structure of the side-chain Adda, open the cyclic structure, or fully oxidize and decompose the toxins into carbon dioxide (Wang 2019). Due to its high-efficiency, cost effectiveness, and environmental friendliness, biodegradation has drawn specific attention as a practical and affordable way to remove MC-LR from the environment (Li et al. 2017).

The MlrABCD degradation pathway is the most effective degradation mechanism among all the established MC-LR detoxification pathways (Dexter et al. 2021). The enzyme MlrA cleaves cyclic MC-LR into linearized MC-LR, MlrB breaks down linearized MC-LR into tetrapeptide, and MlrC breaks down linearized MC-LR and tetrapeptide hydrolyzed to Adda. These are the identified pathways for the degradation of algal toxins (Bourne et al. 1996; Dziga et al. 2012a; Dziga et al. 2012b). In a prior investigation, we discovered that adda in *Sphingopyxis* sp. YF1 is transformed into phenylacetic acid (PAA) by transaminase and β-oxidase, and PAA was subsequently broken down and ultimately transformed into carbon dioxide via the tricarboxylic acid cycle (Wei et al. 2023).

Despite the progresses that have been made in MCs degradation, practical application of biodegradation necessitates consideration of the impact of real-world environmental factors on MCs degradation. MCs degradation may be affected by coexisting contaminants. Studies have demonstrated that heavy metals and MCs often coexist in water bodies such ponds, lakes, reservoirs, and rivers (Cao et al. 2018; Wei et al. 2020; Cao et al. 2020). Cadmium is a significant environmental hazard in aquatic environments. Several domestic waters, including Taihu Lake and the Dongting Lake, were found to be simultaneously polluted by cadmium and MCs (Wei et al. 2019; Wu et al. 2006; Li et al. 2011; Dai et al. 2017). Therefore, it is of great significance to understand the effect of cadmium on the degradation of MCs for evaluating the feasibility of biodegradation practice.

In this experiment, *Sphingopyxis* sp. YF1 was used to study the efficiency of MC-LR degradation and other indexes under different cadmium levels. Scanning electron microscopy (SEM) and transmission electron microscope (TEM) was carried out to analyze the morphological changes and membrane damage in bacterial cells upon exposure to different cadmium levels. The cellular energy metabolism was evaluated by measuring the intracellular ATP content. The generation of MDA as a result of oxidative stress was studied and estimated. RNA was extracted from YF1 under different cadmium levels, and qRT-PCR was used to detect the expression differences of key functional genes (*mlrA*, *pAAase, EAO27_RS08960*) of YF1 under different conditions.

2. Materials and Methods

2.1 Experiment Materials and Instruments

Materials used in this study include MC-LR with a mass fraction of ≥ 95% (Qingdao Prebon Bioengineering Co. , Ltd.), NB medium (Sinopharm Chemical Reagent Co. , Ltd.), and various chemicals such as cadmium chloride, hydrochloric acid, sodium hydroxide, glutaraldehyde, and anhydrous ethanol (Shanghai McLean Biochemistry Science and Technology Co. , Ltd.). The equipment employed comprises an enzyme labeling instrument (Berten Instruments, Ltd. , USA), ultra-high-performance liquid chromatography (Voltaren, Shanghai), a scanning electron microscope (ZEISS), a transmission electron microscope(HITACHI) an ultra-micro UV spectrophotometer (MIULAB ND-100), and a gradient PCR instrument (Eppendorf, Germany). Assay kits used include ATP content, lactate dehydrogenase activity, malondialdehyde content (all from Beijing Soleilbao Technology Co. , Ltd.), and RNA extraction (OMEGA).

2.2 Bacterial strain and chemicals

Sphingopyxis sp. YF1, a bacterium highly proficient in degrading MC-LR, was isolated from water samples collected from Taihu Lake (Yang et al. 2020). The strain is routinely cultivated in NB liquid medium at 30℃ in a shaken flasks oscillated at 180 rpm. MC-LR (Qingdao Prebon Bioengineering Co. , Ltd.) with a purity of 95% was purchased and then stored at - 20 ℃ .

2.3 MC-LR degradation experiments

To assess the impact of cadmium on MC-LR degradation by strain YF1, bacterial culture at logarithmic growth stage (OD_{600}=0. 7) was centrifuged at 5000 ×g for 5 min to collect cells. The harvested cells were then washed twice before being resuspended in sterilized Tris medium supplemented with 1 mg/L MC-LR. The components of Tris medium are (/L): Tris-base 6. 06 g, NaCl 4. 68 g, KCl 1. 49 g, NH_4Cl 1. 07 g, Na_2SO_4 0. 43 g, $MgSO_4 \cdot 6H_2O$ 0. 2 g, $CaCl_2 \cdot 2H_2O$ 0. 03 g, $Na_2HPO_4 \cdot 12H_2O$ 0. 02 g, pH adjusted to 7. 2 ± 0. 2. Cadmium at concentrations of 0. 25 and 4 mg/L were added to the solutions. A control group was set without the addition of cadmium. To evaluate whether cadmium can cause MC-LR degradation, an experiment was conducted in the presence of same content of MC-LR and 4 mg/L cadmium but in the absence of the bacterium. Samples were centrifuged at 12000 ×g for 15 min at 4°C, and the supernatant was subjected to UPLC analysis to measure the remaining MC-LR. UPLC conditions were as follows: the mobile phase consisted of a methanol/water mixture (63/37, v/v) with 0. 05% trifluoroacetic acid. UV detection was performed at a wavelength of 238 nm. The flow rate was 0. 2 mL/min, with an injection volume of 2 μL.

2.4 Electron microscopy

Cells of YF1 were harvested at 180 min under varying cadmium concentrations. Cells were then fixed in 2. 5% glutaraldehyde fixative for 24 hours, dehydrated using a gradient of ethanol concentrations (30%, 50%, 70%, 90%, and 100%), and subsequently vacuum-dried for 4 hours. After even grinding, the samples were gold-sputtered and observed under a scanning electron microscope (Carl Zeiss EVO, Oberko-

chen, Germany). and a transmission electron microscope (Hitachi HT7800, Tokyo, Japan) for morphological analysis to observe the effects of different cadmium concentrations on the YF1 strain.

The Staphylococcus aureus precipitate was collected, washed three times with PBS, re-suspended, and dissolved by adding 2 mL of 2. 5% glutaraldehyde. The solution was fixed for 10 h in a refrigerator at 4°C. The solvent was removed by washing with PBS, and the bacteria were resuspended in PBS. The treated bacterial suspension was dropped onto the metal copper mesh and allowed to stand for 5 min. The excess water on the edge of the copper mesh carrier was sucked off with filter paper, stained with 2% phosphotungstate acid for 30 s, and the samples were placed in the vacuum system of TEM for microscopic examination

2.5 Biochemical characteristics

Cell membrane permeability was determined using the lactate dehydrogenase (LDH) method (Ji et al. 2023). Samples at different time points were withdrawn and centrifuged at 10, 000 ×g for 10 min. LDH content in the supernatant was quantified using a LDH Activity Assay Kit (Solarbio Biotechnology Co. , LTD. , Beijing).

For ATP content measurement, cells of strain YF1 were harvested at different time points. ATP content in cells was detected using the ATP Content Assay Kit as per the manufacturer's instructions (Solarbio Biotechnology Co. , LTD).

For determination of MDA content, cells of strain YF1 were harvested at different time points.

Bacterial lysate was prepared by adding the extraction reagent and subjecting the bacteria to ultrasonic disruption (100W, 3 s on, 10 s off, repeated for 30 cycles in an ice bath). The lysate was then centrifuged at 8000 ×g for 10 min at 4°C, and the supernatant was stored on ice. MDA content was quantified using the MDA Assay Kit (Solarbio Biotechnology Co. , LTD).

2.6 Quantitative real-time PCR

Bacterial solution was withdrawn and cells of strain YF1 was collected by centrifugation at 8000 ×g for 10 min at 4℃ . RNA was extracted using the E. Z. N. A. Bacterial RNA Kit (Omegabiotek, Norcross, USA), and then reverse transcribed into cDNA using the HiScript Ⅱ Q RT SuperMix, (Vazyme, Nanjing, China). The expression of genes involved in MC-LR biodegradation of strain YF1 were measured using quantitative real-time PCR (qPCR) according to the $2^{-\Delta\Delta Ct}$ method. The 16S rDNA gene was used as an internal reference. The primers are listed in Table 1.

Table 1. Primer sequences of target Genes.

Target gene	Sequence(5'—3')
16S rDNA	F:CCTTACCAGCGTTTGACATCC R:CCTTAGAGTGCCCAACTGAATG
mlrA	F:TCGCGCTCTTATCGTAACC R:AGGCGGACGTAGATGAATG
pAAase	F:TCCCATTCACTTGCTGCCAT R:TCGCGGTGATTGCTTACACT
EAO27_RS08960	F: GTGCGATGCTCGACGGCAAGA R: CATATTCAGCCTTCAGCGACT

2.7 Statistical Analysis

SPSS 26. 0 was utilized to generate data files and perform statistical analysis. Independent samples from the two groups were compared using a *t*-test, with statistical significance set at $p < 0.05$ (two-tailed).

3. Results and Discussion

3.1 Impact of cadmium on the degradation of MC-LR by strain YF1

Figure 1 illustrates the effects of cadmium on the degradation of MC-LR. In the absence of *Sphingopyxis* sp. YF1, cadmium at a concentration of 4 mg/L did not decrease MC-LR content, indicating cadmium does not lead to the degradation of MC-LR. At 30 min, the MC-LR biodegradation rates in the presence of 0. 25 mg/L and 4 mg/L cadmium were 10. 28% and 14. 33%, respectively, which were significantly lower than that in the control group (23. 26%). At this time point, the presence of 0. 25 mg/L and 4 mg/L cadmium reduced biodegradation efficiencies by 55. 8% and 38. 39%, respectively.

After treatment for 180 min, the MC-LR degradation rate in the control was 54. 3%, confirming the bacterium's high capacity for MC-LR degradation. The MC-LR degradation rates in the presence of 0. 25 mg/L and 4 mg/L cadmium were 51. 03% and 41. 89%, respectively (Figure 1). Compared to the control, 4 mg/L cadmium significantly hindered MC-LR degradation, with degradation efficiencies reduced by 19. 71%. These results suggest that high concentration of cadmium significantly decreased MC-LR biodegradation rates, while low concentration of cadmium only affected MC-LR biodegradation during the early stage.

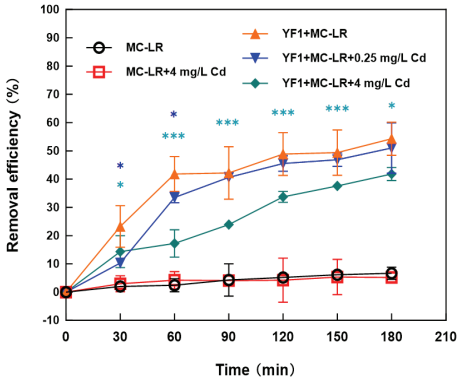

Figure 1. Degradation rate of MC-LR by strain YF1 in the presence and absence of cadmium.

3.2 Effects of cadmium on the morphology of strain YF1

Scanning electron microscopy (SEM) was utilized to examine the effect of cadmium on the morphology of strain YF1 cells. The morphologies of cells in the presence of 0. 25 mg/L cadmium and in the absence of cadmium were similar (Figure 2A and 2B). However, aggregation of cells was observed at a cadmium concentration of 4 mg/L (Figure 2C). On adverse conditions, microorganisms respond to stress by secreting extracellular polysaccharides (EPS) (Ma et al. 2024). Wang et al. (Wang et al. 2024) demonstrated that bacteria employ EPS to facilitate self-aggregation. Therefore, the aggregation of YF1 cells may be a self-protection mechanism under 4 mg/L cadmium stress. Transmission electron microscopy (TEM) analysis demonstrated that YF1 cells in the control group exhibited smooth and intact cell profiles, whereas exposure to cadmium led to visibly wrinkled cell walls (Figure 2D-2F). These findings suggest cadmium affected cell morphology of strain YF1, especially at high cadmium concentration. The morphology of cells can be influenced by various heavy metals. For example, Cr(III) can cause *B. subtilis* cells to change from thin and elongated to short and thick(Fathima and Rao 2017).

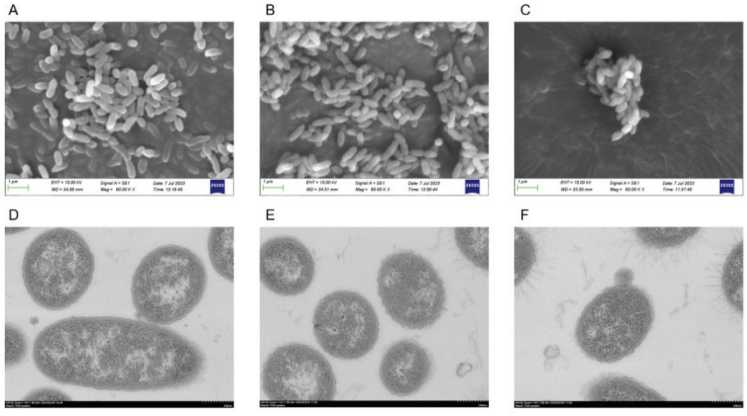

Figure 2. SEM and TEM analyses of *Sphingopyxis* sp. YF1 after treatment with different concentrations of cadmium.

A, SEM image of the control group, B, SEM images of the 0. 25 mg/L cadmium-treated group, and C is the SEM image of the 4 mg/L cadmium-treated group; D, TEM image of the control group, E, TEM image of the 0. 25 mg/L cadmium-treated group, and F is the TEM image of the 4 mg/L cadmium-treated group.

3.3 Effects of cadmium on cell membrane permeability

Lactate dehydrogenase (LDH) is an intracellular enzyme. It can be released into the extracellular space if cell membrane permeability increases. Therefore, measuring LDH content in the culture medium provides an index for cell membrane permeability (Ji et al. 2023). Figure 3 illustrates the variations in extracellular LDH content under different concentrations of cadmium. The results showed that strain YF1 exhibited significantly higher levels of extracellular LDH when exposed to 0. 25 mg/L and 4 mg/L cadmium compared to the control group, suggesting that cadmium can increase cell membrane permeability. The cell membrane serves as the primary defense mechanism against external pollutants, facilitating material transport, energy conversion, and information exchange with the external environment (Li et al. 2024). Elevated permeability and damage to the cell membrane may affect its ability to degrade MC-LR and perform other physiological functions. These results in Figures 2 and 3 suggest that cadmium may cause damages to cell wall and membrane structures, and these damages may further lead to the

decreased MC-LR biodegradation, as is shown in Figure 1.

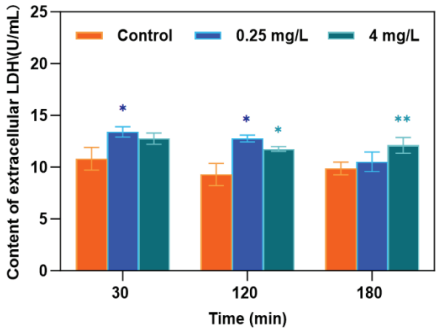

Figure 3. Extracellular LDH content of strain YF1 in the presence and absence of cadmium.

3.4 Effects of cadmium on energy metabolism of strain YF1

Figure 4 illustrates the variations in intracellular ATP content in YF1 cells exposed to different concentrations of cadmium. At 30 min, strain YF1 exhibited significantly higher intracellular ATP levels than the control group under cadmium treatment at 0. 25 mg/L and 4 mg/L. However, at 180 min, there were no significant changes in intracellular ATP content across all cadmium-treated groups. These findings suggest that YF1 cells may increase ATP synthesis in response to cadmium stress during the early stage of degradation (30 min). This ensured the progression of metabolic activities, which may facilitate cells' resistance to cadmium, demonstrating the strain's adaptability to cadmium stress.

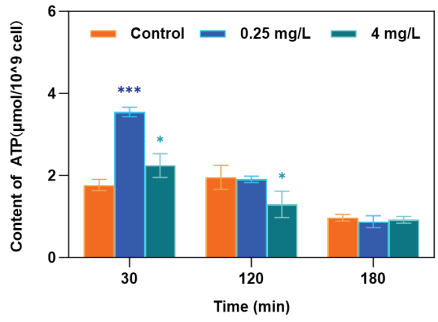

Figure 4. Intracellular ATP content of strain YF1 in the presence and absence of cadmium.

3.5 Impact of cadmium on oxidative stress in strain YF1

MDA serves as an indicator of lipid peroxidation intensity and is commonly regarded as a key marker of reactive oxygen species (ROS) production in organisms (Qian et al. 2012). The MDA content in the 0. 25 mg/L cadmium treatment group was not significantly different from the control group at all time point (Figure 5), indicating lower cadmium stress was less toxic to strain YF1 and did not result in noticeable oxidative damage. Conversely, the intracellular MDA content significantly increased in the presence of 4 mg/L cadmium, especially at 120 min and 180 min. These findings indicate high concentration of cadmium caused oxidative stress in cells of strain YF1. Some studies have found that Cu (I) and Cu (II) could catalyze the production of ROS(Osman and Cavet 2008; Giner-Lamia et al. 2014). Ni toxicity leads to an increase in the number of oxidative stress-related defense proteins in *Pseudomonas putida*(Cheng 2009). The oxidative damage to YF1 was significantly exacerbated at high concentrations of cadmium, thereby resulting in a more pronounced cellular injury and consequently leading to a more substantial decline in the degradation rate of MC-LR.

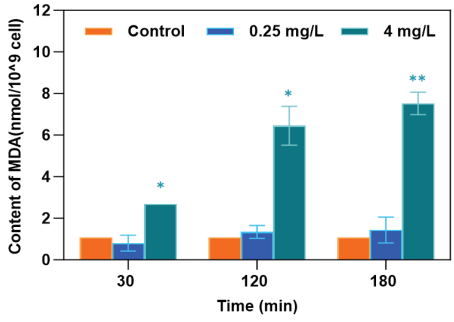

Figure 5. Effects of cadmium on the intracellular MDA content of strain YF1.

3.6 Effects of cadmium on the expression of critical functional genes

The expression of key genes *mlrA* and *pAAase* that are associated with MC-LR biodegradation was investigated in the presence and absence of cadmium at 180 min. As shown in Figure 6, 0. 25 mg/L and 4 mg/L cadmium-treated groups exhibited no significant difference in the expression of *mlrA* and *pAAase* compared to the control group. These suggest that cadmium may not affect MC-LR degradation by reducing the expression of degrading-associated genes. However, Barranwal et al. (Barranwal et al. 2022) found that in the presence of algal organic matter and humic substances, the biodegradation rate of MC-LR decreased by 1. 6 times and 3. 4 times, respectively, and the expression of its degrading genes also decreased. This indicates that different factors affect MC-LR biodegradation via different mechanism.

However, the expression of the gene encoding the ATP synthase B subunit was notably up-regulated in the 4 mg/L cadmium-treated group, exhibiting a 2. 12-fold increase compared to the control group. This may be due to that cells of YF1 need to produce more energy to cope with the stress under high cadmium content. However, the ATP content at 180 in the presence of 4 mg/L cadmium was not higher than in the control (Figure 4). This might be due to that ATP needs to be consumed quickly to respond to heavy metal stress.

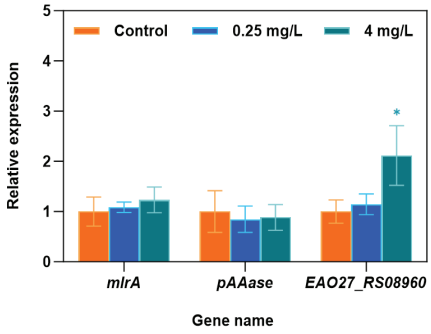

Figure 6. The expression of various genes after treatment with different concentrations of Cd.

In summary, (1)Cadmium can cause various damage to cells, which affects the degradation of MC-LR, which is the main mechanism, but not by down-regulating the expression of degradation-related genes;(2)The degree of damage caused by low and high concentrations of cadmium is different, so the effect on MC-LR degradation is also different. This study revealed the effect and mechanism of cadmium on the biodegradation of MC-LR, which could provide foundation for the practice of MC-LR biodegradation

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Frist author: Fei Yang, Professor, E-mail: phfyang@csu. edu. cn

*** Corresponding author:** Tangjian Peng, E-mail: jianshen. yixiao@163. com