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# Whole-cell and Pure Enzyme-catalyzed Degradation of Microcystins: A Comparative Study of the Efficacy of Heterologously Expressed mlrA Gene

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**Abstract:** ***The increasing occurrence of cyanobacterial blooms, driven by factors such as water eutrophication and climate warming, has raised concerns due to associated environmental pollution and health risks. Among the toxins produced by these blooms, microcystins, particularly Microcystin MC-LR, pose significant threats to ecosystems and human health. This study investigates the efficiency of degrading microcystins, particularly Microcystin MC-LR, using two catalytic methods: whole-cell and pure enzyme catalysis, both relying on the mlrA gene. The research successfully engineered an Escherichia coli strain with the capability to degrade MC-LR, achieving remarkable results with the whole-cell catalysis approach. Within a 24-hour period, the concentration of MC-LR dropped from 5 μg/mL to 0.681 μg/mL, demonstrating an efficiency of 84%. In contrast, enzymatic degradation yielded less satisfactory outcomes, degrading only 100 ng/mL within the same timeframe. The whole-cell catalysis approach exhibits clear advantages, including cost-effectiveness, making it a promising option for industrial applications. This technology holds potential for safeguarding water resources and public health by addressing algal toxin contamination.***

**Keywords:** Microcystins, Catalytic degradation, mlrA gene, Escherichia coli.

## 1. INTRODUCTION

In recent years, the occurrence of cyanobacteria blooms has become a recurring issue, attributed to the eutrophication of water bodies and the gradual warming of the climate. This environmental phenomenon has drawn significant attention from scientists across various disciplines due to the associated environmental pollution and potential health hazards for humans. The primary culprits behind these cyanobacteria blooms are Microcystis and Anabaena [1]. When a cyanobacterial bloom outbreak occurs, these harmful cyanobacteria generate toxic secondary metabolites known as microcystins (MCs) within their cells. These microcystins can be released into the water body when the algal cells die or rupture, resulting in substantial environmental pollution issues [2].

MCs is a cyclic heptapeptide hepatotoxins [3], known for their significant biological toxicity. These toxins can enter the human body through ingestion, leading to a range of health problems. Effects may include diarrhea, nerve paralysis, liver damage, and in severe cases, poisoning or even death. Animal experiments have demonstrated that MCs can impact the development of mouse fetuses by crossing the placental barrier, resulting in hepatitis and renal damage in fetal mice. Additionally, MCs have been identified as carcinogens linked to liver cancer, and they may also affect liver function in children. Notably, MCs can synergize with hepatitis viruses and aflatoxins, compounding the risk of liver cancer [4].

At present, a variety of natural organisms with the ability to degrade MCs have been found, encompassing microorganisms, aquatic plants, zooplankton, and more. These discoveries offer valuable biological tools for managing and remediating MCs pollution. For instance, Pseudomonas putida has demonstrated the remarkable ability to not only lyse Microcystis cells but also degrade MCs. Astonishingly, it utilizes MCs as its sole source of carbon, nitrogen, and energy to fuel its own growth [5]. Furthermore, there are engineered biotechnological treatment methods commonly employed in the prevention and control of cyanobacterial blooms, such as biological filters and constructed wetlands. However, when compared, microbial degradation of MCs emerges as a more efficient and environmentally friendly approach, contributing to the preservation of ecosystem stability [6].

Microorganisms can reduce or lose the toxicity of MCs by changing the structure of the Adda active group on the side chain of MCs or opening the ring structure. Bourne et al [7] first discovered from Sphingomonas sp. that the microcystinase encoded by the gene mlrA can catalyze the degradation reaction of MCs. MCs can decompose into

polypeptide compounds under the action of mlr gene cluster. Based on this, this paper aimed at the mlrA gene, and wanted to explore the comparison of the degradation efficiency of MCs by whole-cell catalytic degradation and enzymatic degradation of the gene. This study can provide some reference value for the utilization of mlrA gene.

## 2. MATERIALS AND METHODS

### 2.1 Cloning and plasmid construction of mlrA

We initially acquired the mlrA gene sequence from Sphingomonas sp. (ACM-3962) through the NCBI database. Subsequently, we enlisted the services of a biological company to synthesize a codon-optimized version of the mlrA gene sequence. We then devised specific primers for both the gene sequence and the vectors, namely the pcs-27 vector and the pet-dute1 vector, to facilitate the cloning process.

To prepare the vectors for cloning, we employed appropriate enzyme cutting sites to linearize them. These linearized vectors were then subjected to ligation with the cloned gene fragment. This intricate process culminated in the successful construction of two plasmids: the pcs-27 plasmid and the pet-dute1 plasmid, both of which now carry the mlrA gene.

### 2.2 Transformation of Escherichia coli

Both pcs-27 transformation of Escherichia coli BW25113(F') and pet-dute1 transformation of Escherichia coli BL21 were transformed by electroporation. Transfer the Escherichia coli competent cells after ice bath and the plasmid constructed above to the electrotransformation cup, tap the electrode cup gently to make the mixture enter the bottom of the electrode cup, turn on the electrotransfer instrument, and adjust the voltage to 2.1KV. After the electric shock was over, 800 μL of LB liquid medium was quickly added to the electric shock cup, and the resistant plate was coated after recovery. After overnight culture, clones were picked to obtain transformed Escherichia coli.

### 2.3 Whole-cell catalysis

The E. coli Bw25113(F') containing the Pcs27-mlrA plasmid was picked into a test tube, and then the E. coli was inoculated into a shake flask. Wait until OD600 = 0.6 before adding IPTG for induction. When OD=3-5, precipitate and collect E. coli. Then use M9 medium to resuspend the bacterial pellet. Add substrate MC-LR (cyanotoxin) to the suspension to a final concentration of 5 μg/mL, then transfer it to a 24-well plate to continue culturing. Samples were taken from the 24-well plate every three hours. After the extracted samples were centrifuged, the precipitate was discarded and the clear liquid above was taken. Freeze-dry in a freeze dryer, dissolve in ethanol solution, and after fully dissolved, add water 1:1 and mix evenly. Finally, filter the mixed culture solution with a filter membrane with a diameter of 0.22m, and put the sample into a liquid phase vial for later use.

### 2.4 Protein purification and in vitro catalysis

Escherichia coli BL21 containing the pet-dute1-mlrA plasmid was cultured and IPTG was added to induce the expression of the target protein. After expression of the target gene, protein purification is required. In this process, His-Tag protein purification and MBP column purification were used respectively (Figure 1 and Figure 2). Then, the BCA protein quantification method was used to determine the concentration of the purified target protein. Finally, SDS-polyacrylamide gel electrophoresis was used to detect the molecular weight and purity of the purified protein. Take 3 parts of 1ug of the target protein and add the substrate MC-LR (algae toxin) to a final concentration of 600 ng/mL. Samples were collected at the time points of 4, 8, 12, and 24 hours of incubation for subsequent detection.

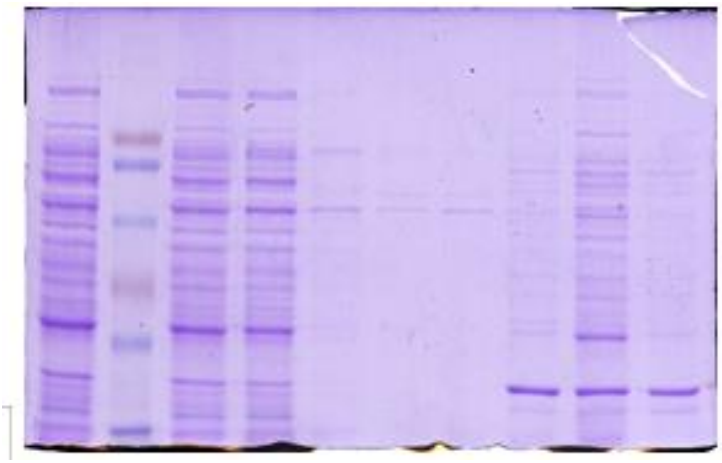

**Figure 1:** Electrophoresis gel map of pET-DuetI-His-Tag-mlrA enzyme

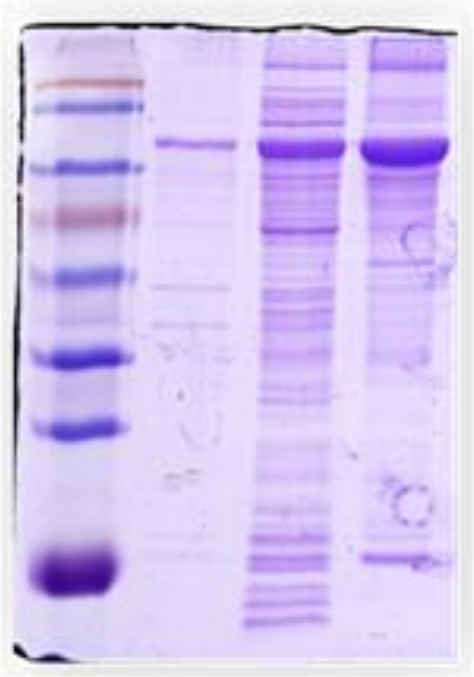

**Figure 2:** Electrophoresis gel map of pET-Duet1-mbp-sp.mlrA enzyme

**2.5 Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis**

**Instruments:**

For this analysis, we utilized the Agilent 1290-AB SCIEX 5500QTRAP tandem triple quadrupole liquid chromatography mass spectrometer. The chromatographic separation was performed on a ZORBAX Eclipse Plus C18 chiral column (2.1×50mm, 1.8-micron particles). Additionally, a 1 mL pipette was used for sample handling.

**Reagents:**

We employed high-quality reagents, including methanol (chromatographic grade), formic acid (chromatographic grade), acetonitrile (chromatographic grade), and a microcystin MC-RR standard with a purity exceeding 99%.

**Chromatographic Conditions:**

Mobile Phase: The mobile phase consisted of two components:
Mobile Phase A: Formic acid aqueous solution with a volume fraction of 0.05%

Mobile Phase B: Acetonitrile

**Gradient Elution Conditions:**

0-2.5 minutes: Mobile phase A with a volume fraction of 80%
2.5-3 minutes: Mobile phase A with a volume fraction of 10%
3-3.5 minutes: Mobile phase A with a volume fraction of 10%
3.5-7 minutes: The volume fraction reverts to 80% for mobile phase A

**Flow Rate:**

The flow rate was set at 200 μL/min.

**Column Temperature:**

To maintain consistency, the column temperature was maintained at 25°C throughout the analysis.

**Injection Volume:**

A fixed injection volume of 10 μL was used for the samples.

**Mass Spectrometry Conditions:**

**Ion Source:** The ion source employed for this analysis was an electrospray ionization source (ESI).

**Scan Mode:** The analysis was conducted in positive ion scan mode.

**Collection Method:** Multiple Reaction Monitoring (MRM) was employed to gather data with precision and specificity.

## 3. RESULT

### 3.1 Whole-cell catalytic degradation of MCs by mlrA

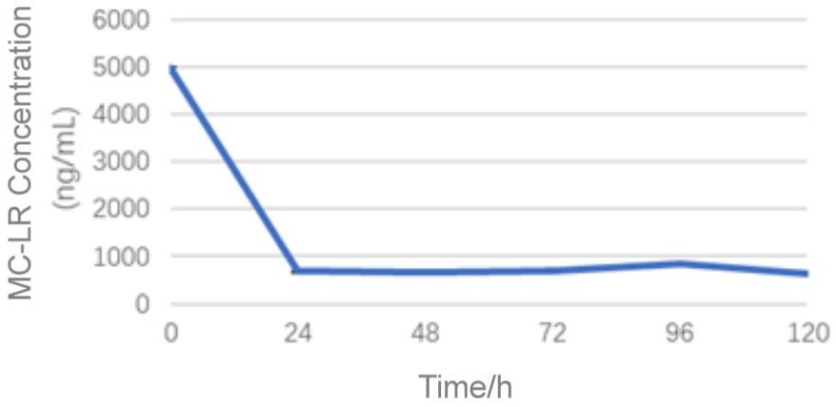


**Figure 3:** Degradation efficiency within 24 hours

As illustrated in Figure 3, our study employed a whole-cell catalysis approach, wherein the complete Escherichia coli BW25113(F') strain, carrying the mlrA gene, was utilized to facilitate the quantitative degradation of MC-LR. The results of this approach were nothing short of impressive. Within a mere 24-hour timeframe, the concentration of MC-LR in the solution plummeted from an initial 5 mg/mL to a remarkable 0.8 mg/mL. Consequently, the degradation efficiency achieved through this whole-cell catalytic method within this 24-hour period amounted to an impressive 84%.

### 3.2 mlrA enzyme catalyzes the degradation of MCs in vitro

When we conducted degradation experiments using purified mlrA enzyme, the results were not as favorable. As depicted in Figure 4, the enzyme's impact on MC-LR degradation became noticeable starting from the 4th hour, and it took a considerable 12 hours to reach its maximum degradation capacity. However, at the 24-hour mark, an unexpected trend emerged: the concentration of MC-LR substrate did not decrease but actually increased. This anomaly may be attributed to potential errors arising from water evaporation during the incubation process.

In contrast, the concentration of the MC-LR substrate in the control group, the empty medium, exhibited fluctuations within a certain range without displaying any statistically significant trends. Ultimately, after 24 hours, the purified enzyme had only managed to degrade 100 ng/mL of the substrate MC-LR, resulting in a degradation efficiency of just 16.6%. These findings underscore the limitations of using purified mlrA enzyme and highlight the superior performance of the whole-cell catalysis approach previously discussed.

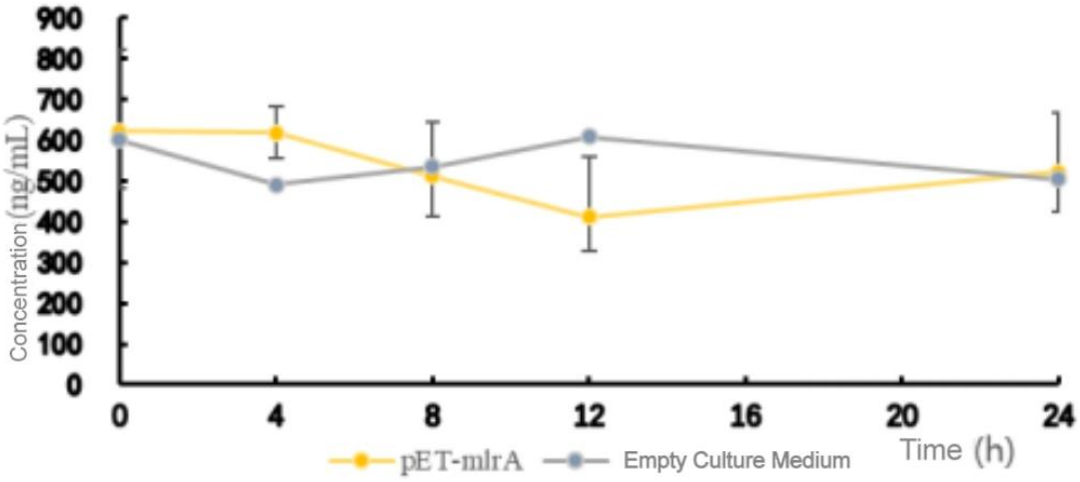


**Figure 4:** Enzymatic degradation of MC-LR

## 4. DISCUSSION

Our study marks a successful endeavor in the utilization of microorganisms for assessing the degradation efficiency of algal toxins stemming from algal blooms. We have not only established a method for detecting trace algal toxin MC-LR but also conducted experiments utilizing two distinct approaches.

In our first achievement, we successfully engineered an Escherichia coli strain with the capability to degrade Microcystin MC-LR. This innovative approach yielded impressive results, as it managed to reduce the concentration of 5 μg/mL MC-LR to just 0.681 μg/mL within a mere 24-hour span. However, our attempts at enzymatic degradation of the algal toxin did not yield as promising outcomes, with only 100 ng/mL being degraded within the same timeframe.

Several factors likely contributed to these differential outcomes. Firstly, during the enzyme purification process, factors such as experimental procedures or environmental conditions may have inadvertently influenced the enzyme's structure and activity, subsequently affecting its efficacy in degrading MC-LR. Secondly, the whole-cell catalysis method demonstrated its inherent advantages. Whole-cell catalysis leverages a complete multi-enzyme system, capable of orchestrating cascade reactions, all while eliminating the need for the laborious enzyme purification process. This approach, therefore, circumvents any adverse effects on enzyme activity, leading to its superior performance [8].

## 5. CONCLUSION

In conclusion, this study represents a comprehensive examination of the comparative efficiency in degrading microcystins using two distinct catalytic methods: whole-cell and pure enzyme catalysis, both hinging on the mlrA gene. Our experimental findings underscore the successful creation of an Escherichia coli strain with the remarkable capability to degrade Microcystin MC-LR. In particular, the whole-cell catalysis approach exhibited exceptional prowess in this endeavor, efficiently reducing the concentration of MC-LR from 5 μg/mL to a mere

0.681 μg/mL within a 24-hour timeframe. In contrast, the enzymatic degradation of algal toxins, although attempted, yielded less satisfactory results, with only 100 ng/mL being degraded within the same time frame.

The superiority of the whole-cell catalyzed degradation method is unmistakable. Beyond its evident efficacy, it boasts the additional advantages of cost-effectiveness, making it a viable choice for widespread industrial applications. This technology holds the potential to be harnessed in the industrial-scale production of E. coli strains tailored for degrading Microcystin MC-LR. Such applications hold significant promise, particularly in scenarios where the presence of algal toxins produced during cyanobacterial blooms poses a threat to water resources. By harnessing the capabilities of E. coli to neutralize these toxins, this technology can contribute significantly to safeguarding not only water resources but also the well-being and property of the people who rely on them. It is a testament to the intersection of science and practical solutions in addressing pressing environmental and public health concerns. The study was conducted under controlled laboratory conditions. Future research should assess the effectiveness of these catalytic methods in real-world environmental settings, considering factors such as water quality, temperature, and pH.

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