

Biodegradation of microcystin-RR and -LR by an indigenous bacterial strain MC-LTH11 isolated from Lake Taihu

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Abstract: The indigenous bacterial strain MC-LTH11 with the capability of degrading microcystin-RR (MC-RR) and microcystin-LR (MC-LR) was successfully isolated from Lake Taihu. The bacterium was identified as *Stenotrophomonas* sp., which possessed a *mlrA* gene. The MC-LTH11 thoroughly degraded MC-RR and MC-LR with the initial concentration of 37.13 mg/L and 18.49 mg/L, respectively, in the medium containing crude microcystins extract within 6 d. The degradation rates were affected by temperature, pH, initial MCs concentration and the kinds of media. Additionally, the bacterial strain MC-LTH11 also degraded thoroughly microcystins in the water body of Lake Taihu within 1 d. These results suggest that the *Stenotrophomonas* sp. MC-LTH11 has the capacity to bioremediate water bodies contaminated by microcystins, and may contribute to the degradation of microcystins after the outbreak of harmful cyanobacterial blooms in Lake Taihu.

Key words: water treatment; biodegradation; microbiology; microcystins; high performance liquid chromatography (HPLC)

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Lake Taihu is situated on the ancient Yangtze River delta with a catchment of 36 500 km², and surrounded by most heavily industrialized cities with almost the highest population density in China^[1]. Due to the massive discharge of industrial wastewater into Lake Taihu in recent years, harmful cyanobacterial blooms (HCBs) occurred frequently in Lake Taihu, which gave a threat to residents' lives and health^[2]. In 2007, the HCBs spread out over 40% of the total area of Lake Taihu, which caused sudden water pollution accidents in Wuxi City^[3-4]. HCBs also produce many kinds of microcystins (MCs), which can cause illness and death in humans^[5]. A guideline value of 1.0 µg/L microcystin-LR (MC-LR) in drinking water was set by the World Health Organization^[6].

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Microcystin-RR (MC-RR) and MC-LR have the same cyclo-(D-Ala-X-D-MeAsp-Z-Adda-DGlu-Mdha-) structure, where X and Z represent variable L-amino acids^[7]. They are stable and resistant to physicochemical and biological factors such as temperature, pH values, sunlight, and enzymes^[8]. Although MCs can be destructed by some conventional and advanced oxidation processes, the biodegradation of MCs by indigenous bacteria can be one of the safest and mildest treatments for removing cyanobacterial toxins from fresh water^[8-9]. To date, only a few bacteria with the ability to degrade MCs have been reported, and most strains appear to be limited to the family *Sphingomonas*^[9].

Bourne et al.^[10] first identified a gene cluster, *mlrA*, *mlrB*, *mlrC* and *mlrD* in strain ACM 3962, which was responsible for the degradation of MC-LR. The most important enzyme *MlrA* coding by the *mlrA* gene breaks off the cyclic peptide of MC-LR to linear MC-LR. Moreover, the toxicity of the degradation products of MC-LR, including the linearized MC-LR and the tetrapeptide, was less than that of MC-LR^[11].

The objectives of this study are to isolate and identify the indigenous MC-degrading bacteria from Lake Taihu, then explore the mechanism of MCs degradation by the bacteria and investigate the capacity of degrading MC-LR and MC-RR by the indigenous bacteria under different conditions.

1 Materials and Methods

1.1 Preparation and analysis of MCs

Authentic MCs were purchased from the Sigma, and crude MCs were extracted from the lyophilized *M. aeruginosa* cells. MCs were identified using the Agilent 1100 high performance liquid chromatography (HPLC). The mobile phase was a combination of Milli-Q water (Millipore, Germany) containing TFA and methanol (Dikma Technology Inc., USA) (45:55, volume ratio), which was set at a flow rate of 1 mL/min. The injection volume was 20 µL at 40 °C for analysis and an absorbance at 238 nm was monitored. The MC-RR and MC-LR in the crude extracts were 46.6 mg/L and 23.1 mg/L, respectively.

1.2 Isolation and identification of MC-degrading bacteria

The sediment sample was suspended in sterilized water and shaken at 120 rev/min for 30 min. The supernatant

was inoculated into a mineral salt medium (MSM) mixed with crude MC extracts. MSM was composed of 1.0 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L KH_2PO_4 , 4.0 g/L K_2HPO_4 , 1.0 g/L NaCl, 20 mg/L CaCl_2 , 5 mg/L FeSO_4 , 5 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 5 mg/L ZnCl_2 , and 0.5 mg/L CuCl_2 . Inocula were subcultured for 4 times. Subsequently, the medium diluted gradually was spread onto the MSM agar plates (2% agar), which also contained crude MCs extract. After cultivating, single colonies were transferred to MSM containing crude MC extracts and cultivated. The capability of degrading MCs was identified by monitoring the remaining MCs in the media, and the MC-degrading strain can be screened out.

Bacterial DNA was extracted by using TIANamp Bacteria DNA Kit (TIANGEN Biotech, Beijing, China). The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using forward primer (5'-AGAGTTTGATC-MTGGCTCAG-3') and reverse primer (5'-TACG-GYTACCTTGTTACGACTT-3'). The PCR was carried out for 35 cycles of 94 °C for 1 min, 55 °C for 1 min and then 72 °C for 2 min. The 16S rRNA gene PCR product was sequenced by the BGI Company, China. The homology of the 16S rRNA gene sequence of the isolates, with reference 16S rRNA gene sequences, was analyzed using the BLAST algorithm in GenBank available in the National Centre for Biotechnology Information (NCBI).

1.3 PCR for *mlrA*

The *mlrA* gene was amplified by PCR using specific forward primer (5'-GACCCGATGTTCAAGATACT-3') and specific reverse primer (5'-CTCCTCCCA-CAAATCAGGAC-3'). The PCR was carried out for 40 cycles of 98 °C for 10 min, 56 °C for 30 s and then 72 °C for 30 min, with an initial denaturation at 95 °C for 5 min and a final elongation at 72 °C for 5 min.

1.4 Batch degradation experiments

The ability of the bacterial strains to degrade MCs iso-

lated from Lake Taihu was examined by inoculating them into the different media: MSM with the crude extract, MSM with the authentic MC-LR, and water body samples collected from Lake Taihu. Before inoculating at a constant condition, the strains were cultured for 48 h with shaking in MSM containing MCs, harvested by centrifugation, and washed twice with 20 mmol/L sodium phosphate buffer (pH = 7.2). The MSM containing MC extracts was employed for evaluating the effect of temperature, pH value and MCs concentrations on the degradation of MCs. All experiments were carried out in duplicate, and bacterial-free medium was employed as a control.

2 Results and Discussion

2.1 Isolation and identification of MC-degrading bacteria

A sediment sample from cyanobacteria-salvage yards at Fudu Port in Lake Taihu was collected and screened. The bacterial strain MC-LTH11 showed a great ability to degrade MC-RR and MC-LR. This strain was aerobic, gram-negative, rod-shaped and produced white-colored colonies on agar medium.

For bacterial identification, the 16S rDNA gene for the isolate MC-LTH11 was amplified. The obtained DNA nucleotide was sequenced by the BGI Company, China and then analyzed using DNA Blast search (NCBI). The analysis of the 16S rDNA sequence reveals that the bacterium belongs to the genus *Stenotrophomonas* species (see Fig. 1), and deposited in the Genbank with an accession number of KC734883. Recent investigations have revealed the isolation of some different MC-degrading bacteria isolated from different China water bodies^[10]. Among these bacteria, the strain EMS isolated from Lake Taihu also belongs to the *Stenotrophomonas* sp., which indicates that *Stenotrophomonas* sp. may be a common MC-degrading bacterial strain and play an important role in natural MCs degradation, in Lake Taihu.

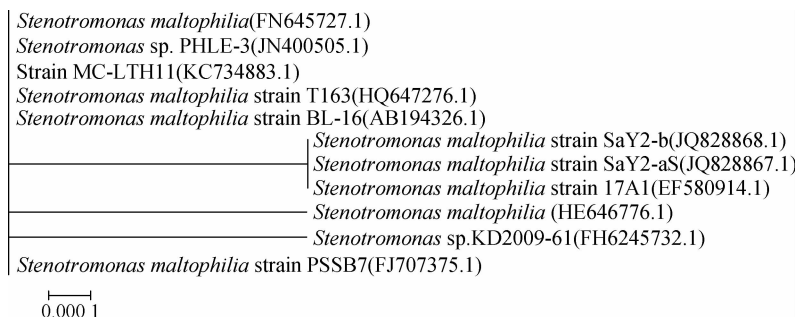
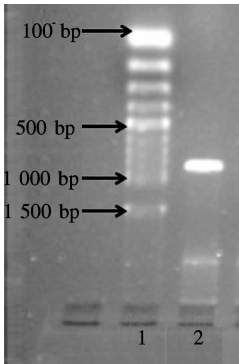


Fig. 1 Phylogenetic tree of *Stenotrophomonas* sp. MC-LTH11

2.2 Detection of *mlrA* gene from MC-degrading bacterium MC-LTH11

DNA extracted from MC-LTH11 and specific oligonucleotide primers were used for PCR targeting the *mlrA*

gene with conditions as described before. The amplification product of *mlrA* (approximately 850 bp) was obtained (see Fig. 2). This result confirms that *mlrA* homologues exist in this bacterial strain.



1—Marker; 2—MC-LTH11

Fig. 2 Detection of *mlrA* gene fragment by PCR in MC-degrading strain MC-LTH11

2.3 MCs degradation in batch experiments

The degradation rates of MCs were significantly affected by temperature. At 37 °C, the MC-RR and MC-LR were completely removed at the highest degradation rates of 6.18 mg/(L · d) and 3.08 mg/(L · d), respectively. Meanwhile, the analogues were degraded at 30 °C and 20 °C in lower rates. The degradation rates of MCs were also affected by pH values. The optimum pH values for degradation were neutral or weak acidic conditions. These results are different from strain EMS, which prefers to degrade MC-RR and MC-LR in alkaline rather than acidic environments^[12].

Fig. 3 shows the highest initial MCs (37.13 mg/L MC-RR, 18.49 mg/L MC-LR) were degraded at the highest

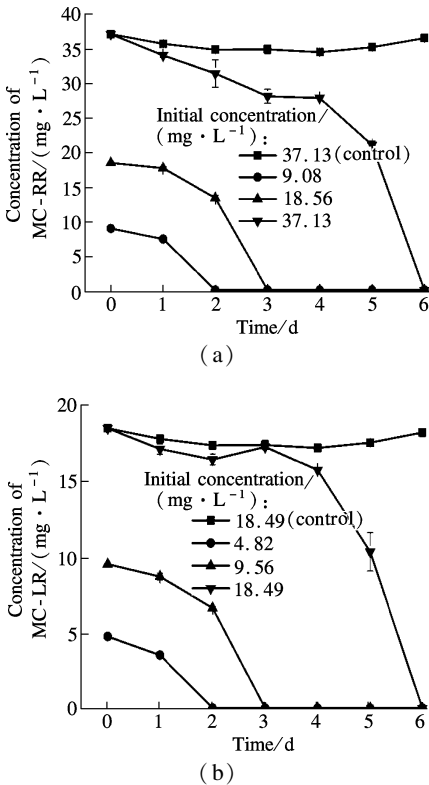


Fig. 3 Effect of MCs concentrations on degradation of MCs. (a) Degradation for MC-RR; (b) Degradation for MC-LR

rates of 6.18 mg/(L · d) and 3.08 mg/(L · d), respectively. When the initial MCs decreased to 18.56 mg/L MC-RR and 9.56 mg/L MC-LR, the degradation rates were 6.19 mg/(L · d) and 3.19 mg/(L · d), respectively. At the lowest initial MCs (9.08 mg/L MC-RR, 4.82 mg/L MC-LR), the rates reduced to 4.54 mg/(L · d) and 2.41 mg/(L · d), respectively. Lag periods were evident prior to MC-LTH11 initiating the degradation of both analogues, and gradually shortened with the decreased analogues concentration.

Many studies reported that a lag phase exists before the initiation of degradation, but it is not clear why this lag phase exists. A possible explanation for the lag phase is that the synthesis of MCs degradative enzymes (microcysrinase) is repressed by some catabolite repressor substrates present in the *M. aeruginosa* extracts^[13]. In this study, both heavy metals and catabolite repressor substrates may exist in MSM with the crude extract, which can lead to the emergence of the demurrage. Nevertheless, once degradation commences after the lag period, both MCs can complete removal quickly.

Fig. 4 shows that authentic MC-LR at 14.41 mg/L was removed directly at a rate of 7.21 mg/(L · d). Compared with the experiment in MSM with the crude extract, the degradation rate increased, and the lag phase disappeared, although the initial concentration of MC-LR was at a higher level.

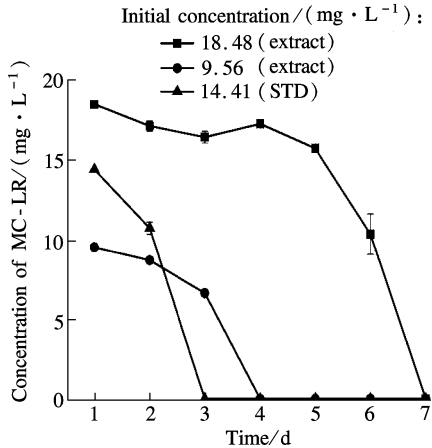


Fig. 4 Degradation of MC-LR in the MSM with the crude extract and authentic MC-LR

MC-LTH11 also performed a good ability of degradation for MCs in the water body collected from Lake Taihu. MC-RR at 3.56 mg/L and MC-LR at 2.04 mg/L were completely removed in 24 h while no losses of MC-RR and MC-LR were evident in control (see Fig. 5). These results confirm that the MC-LTH11 strain may be actively involved in the degradation of MCs during the disappearance of the HCBs in Lake Taihu. Since the application of the biological sand filter embedded with MC-degrading bacteria MJ-PV successfully removed MC-LR and MC-LA from source water^[14], and a bioreactor

using the immobilized B-9^[11] completely degraded MC-RR after 24 h, an effective biofilter with immobilized MC-LTH11 to degrade MC-LR and MC-RR may be our future research.

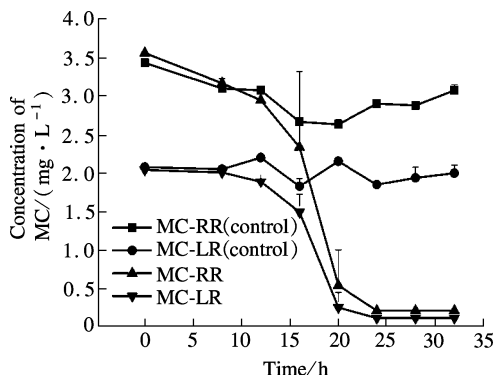


Fig. 5 Degradation of MC-RR and MC-LR in water body collected from Lake Taihu

3 Conclusion

An indigenous MC-degrading bacterium MC-LTH11 isolated from Lake Taihu is identified as *Stenotrophomonas* sp. based on 16S rDNA sequence analysis. A *mlrA* gene involved in MCs biodegradation is detected in MC-LTH11. MC-LTH11 has a capability of MC-degrading in MSM with MCs and body water of Lake Taihu, and the degradation rates are dependent on temperature, pH, initial MCs concentration and media. Therefore, the *Stenotrophomonas* sp. MC-LTH11 has the capacity to bioremediate water bodies contaminated by MCs and may contribute to the degradation of MCs after the outbreak of harmful cyanobacterial blooms in Lake Taihu.

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太湖土著菌 MC-LTH11 的筛选及其对藻毒素-RR 和-LR 的生物降解

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摘要:从太湖中成功筛选出一株能够同时降解微囊藻毒素-RR(MC-RR)和微囊藻毒素-LR(MC-LR)的土著细菌 MC-LTH11. 经鉴定,该菌属寡养单胞菌属,并含有 *mlrA* 基因. 在含有藻毒素粗提物的培养基中,MC-LTH11 能够在 6 d 内将初浓度分别为 37.13 mg/L 的 MC-RR 和 18.49 mg/L 的 MC-LR 完全降解,并且降解速度受到 pH 值、温度、初始微囊藻毒素浓度和培养基种类的影响. 此外,MC-LTH11 能够在 1 d 内完全降解太湖水样中的微囊藻毒素. 研究结果表明,寡养单胞菌 MC-LTH11 具备对微囊藻毒素污染水体的修复能力,并可能是太湖水华爆发后微囊藻毒素降解的重要因素.

关键词:水处理;生物降解;微生物学;微囊藻毒素;高效液相色谱法

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