Isolation of a *Arthrobacter* sp. T11 that Degrades Cyanobacterial Hepatotoxin Microcystin from the Sediment of a Shallow Hypertrophic Lake

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***Abstract*—In present study, we are intrested in establishing a biological removal method by microorganism against the cyanobacterial hepatotoxin microcystion. A native microcystin-degrading bacterium was isolated from a shallow hypertrophic lake, and the biodegradation effect was detected by the LC-MS method. The 16S rDNA gene sequence analysis result indicated that the isolated strain T11 was belonged to the genus *Arthrobacter sp.,* with a highest sequence similarity (98%) with *Arthrobacter* sp. C6 strain. LC-MS result showed that the *Arthrobacter* sp. T11 was able to effectively degrade, at a highest concentration of MC (MC-LR ~ 15μg·L^{-1}; MC-RR ~ 20μg·L^{-1}) in batch experiments using sterilized lake water conditions, with complete removal observed within 10 days.**

Keywords-degradation; microcystin; arthrobacter sp.; hypertrophic lake.

I. INTRODUCTION

In the past decades it is apparently that harmful cyanobacteria blooms (HCB) have adverse effects on artificial and natural freshwater systems in the world [1-4]. Cyanobacteria, such as *Microcystis*, *Oscillatoria, Anabaena and Nostoc species*, produce a group of hepatotoxins called microcystin (MC) [5-6]. Among the 80 structural analogues of microcystin, microcystins-LR (MC-LR) and microcystins-RR (MC-RR) are the two most typical and toxic types which have been shown to affect the hepatopancreatic, digestive, dermal, endocrine and nervous systems in mammals (including humans) [7].

Recent works indicated several bacterial strains, such as *Sphingomonas sp., Sphingosinicella sp., Burkholderia sp., Brevibacterium sp., Arthrobacter sp. and Bacillus sp.*, have been regarded as excellent candidates for microcystin-degrading properties [8-11]. In contract to several chemical treatments, biodegradation methods not only avoid producing carcinogenic substances and other mutagens, but also effectively remove microcystins [12]. Thus, study on microbial degradation seems to be one of the more safety and effectively solutions to remove microcystins.

Lake Taorangting (39°87′N, 116°38′E) is well known as a famous recreational place in China. In recent years, the lake showed a toxic cyanobacteria bloom mainly producing the MC-RR and MC-LR, posing a threat to public humans and animals healthy [13-14]. It is hypothesized that local bacterial community frequently which are often exposed to blue-green blooms MC could degraded MC very well [15]. Therefore, the main purpose of this study was to isolate and identify a native MC-degrading bacterium.

II. MATERIALS AND METHODS

A. Detection of MC

Identification and quantification of MC were performed by LC/MS with Dionex Ultimate 3000 (Dionex, USA), an Ultimate XB-C_{18} column (150mm×4.6mm, 5μm) and a 3200Q TRAP LC/MS/MS Mass system (Aglient, USA). The mobile phase was Methanol (D) and water with 0.1% (v/v) formic acid (A).. The precursor ion *m/z* of MC-LR, RR were 995.6 and 519.8, respectively.

B. Isolation of the MC-Degrading bacteria

The supernatant fluid of sediments taken from Lake Taoranting was inoculated into NA medium plates. Single colonies were transferred to 50ml of NB medium. To investigate degradation of MC, twenty isolated bacterial strains were inoculated (5ml) separately to 50 ml NB medium. The density of isolated bacteria was 1.5×10^6 cell·ml^{-1}. All cultures of bacterial strains were maintained at 30℃ with shaking (120 rpm) in the dark for 5days. The remaining concentrations of MC-LR and MC-RR in the medium were monitored by LC/MS.

C. 16S rDNA Sequencing and phylogenetic

The capable of MC –degrading bacterial strain was identified by 16S rDNA sequence analysis, which was amplified by polymerase chain reaction (PCR) using two universal primers (1F: 5´-AGAGTTTGATCCTGGCTCAG-3´) and (1R: 5´-GGTTACCTTGTTACGACTT-3´). Sequencing steps were carried out at Qsingke Biological Technology Company, China. A BLAST search was performed against homologous sequences in GenBank (NCBI). The 16S rDNA gene sequences of related species with the other of MC-biodegradation bacteria were aligned, and evaluated by 1000 bootstrap replicates using the Neighbour-Joining plot program by MEGA version 6.0[16].

D. Biodegradation of MC

Cyanobaterial bloom samples of the Taoranting Lake could produce MC-LR at 15 μg·L^{-1} and MC-RR at 20 μg·L^{-1}

approximately. The ability of the isolated bacteria strain to degrade MC-LR and MC-RR were examined at initial concentration of 5, 10 and 15 μg·L^{-1} for MC-LR, and 6, 12 and 20μg·L^{-1} for MC-RR, separately. Parallel experiments were conducted using the isolated bacteria, which initial bacteria density was 1.5×10^6 cell·ml^{-1}.The bacterial-free medium was employed as a control in each experiment.

III. RESULTS

A. Isolation of MC-degrading bacteria

A total of 20 isolates were screened for the MC-degrading bacteria from the NB medium. Among the test strains, only one isolate named T11, was capable of biodegrading MC-LR and -RR significantly. The concentrations of MC were also measured by LC/MS. LC/MS revealed that at the beginning of the experiments containing MC-LR (1050 ng·m1^{-1}) and MC-RR (335ng·ml^{-1}). As shown in Fig 1, each sample at the initial stage was considered 100%. After 5 days MC-LR was completely degraded within 5 days (Fig.1 (a)), and almost 98% of MC-RR analyzed from all experiments was absent (Fig.1 (b)).

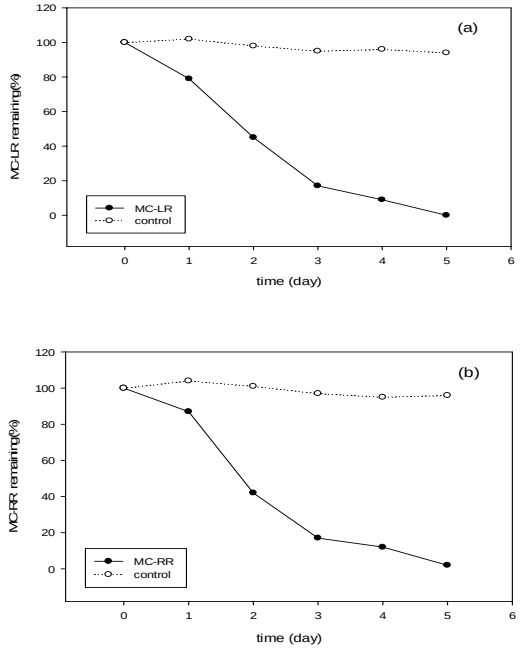

Figure 1. Biodegradation of MC –LR (a) and –RR (b) within five days by the strain T11 isolated from Lake Taoranting.

B. Molecular identification of the isolated bacteria

To confirm the taxonomic and phylogenetic of the isolated MC-degrading strain T11, its partial 16S rDNA gene sequence was identified and compared with the nucleotide sequence using the NCBI-BLAST Database. Analysis of its 16S rDNA sequence analysis indicated that the isolated bacteria T11 belonged to the genus *Arthrobacter* sp. The Neighour-joining phylogenetic tree was constructed by a dataset that consisted of Strain T11 with the other close relative MC-degrading bacteria reported previously (Fig. 2). This revealed isolate T11 branched deeply from a major cluster of MC-degrading bacterial isolates including *Arthrobacter* sp. F1, R4, R1, C6 and *Brevibacterium* sp. F3. It has been suggested that sequence similarity must be above 95% to quality as evidence of a similar species. Thus, it is most likely to confirm that the strain T11 was a member of the genus *Arthrobacter*.

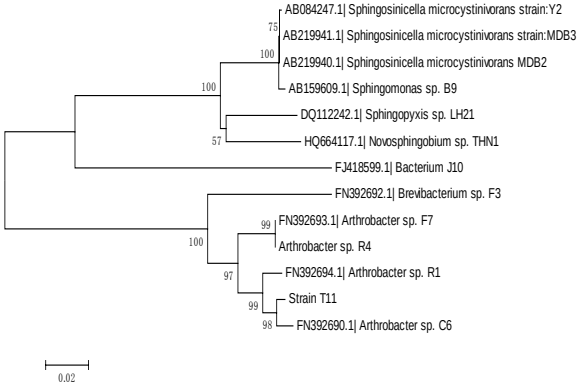

Figure 2. Neighour-joining phylogenetic tree indicting the position of T11.

C. Biodegradation of MC by strain T11

Fig.3. shows the effect of MC-degrading bacterium T11 in sterillised Lake Taoranting water. At higher initial microcystin concentrations (MC-LR ~ 15μg·L^{-1}; MC-RR ~ 20μg·L^{-1}) were completely removed within 10 days, while at the lowest MC concentrations (MC-LR ~ 5μg·L^{-1}; MC-RR ~ 6μg·L^{-1}) with complete removal observed for 4 days. There is no apparent degradation of both microcystins analogues in the control.

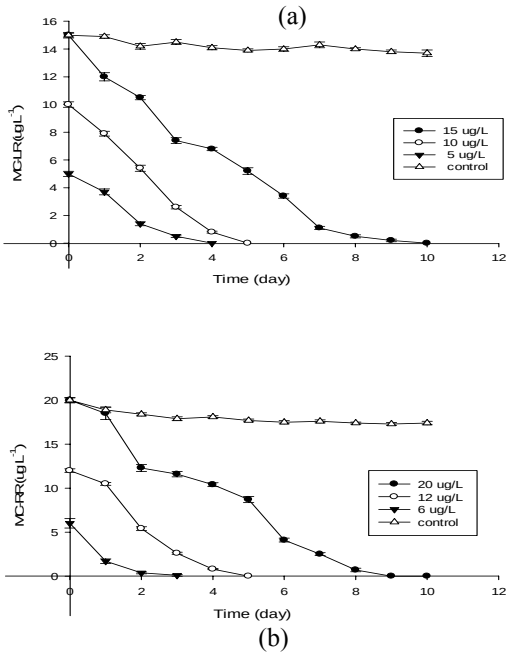

Figure 3. The biodegradation effect of the isolated strain T11.

IV. DISCUSSION

Microsytins pollution is considered to be an important water quality problem especially in urban lakes, because of implicating a serious health problem in water supplies and human and animals' health [17]. According to the previous reports, biodegradation is of one of the essential process for degrading such toxins in natural and artificial eutrophic lakes. However, most of the work on the MC-degrading bacteria focused on MC-LR. There is little information was available on both MC-LR and MC-RR removal by aquatic microorganisms [18-19].

In the present study, an *Arthrobacter* sp. T11 isolated from Lake Taoranting sediment could degrade both MC-LR and MC-RR. Chen *et al*. [20] suggested that the degradation of MC in the presence of lake water with sediments has a significantly faster biodegradation rate than in lake only. This was attributed to its nutritional versatility of growing on a large amount of organic compounds. The results of phylogenetic analysis base on the 16S rDNA gene sequence data showed a homology (98%) between *Arthrobacter* sp. C6 and *Brevibacterum* sp. F3. Kormas *et al*. [21] suggested that *Brevibacterum* sp taxonomically and physiological similar to *Arthrobacter* sp..

The degradation pathway of microcystin-LR has been characterized by *Sphingononas* sp. strain ACM-3962 [22]. They suggested that the *mlr* gene cluster is responsible for MC-LR, and *Mlr* B degrading linearized MC-LR to tetrapetide. Recently, Yan *et al*. [23] reports suggested that first step in the biodegradation of MC-RR by the isolated *Sphingopyxis* sp. USTB-05 was similar to that of MC-LR, begin with the breakage of Adda-Arg bond in the ring of MC-RR. LC/MS profiles of MC-LR and MC-RR by the strain *Arthrobacter* sp. T11 was conducted in this study. Therefore, the mechanism of MC degradation in strain *Arthrobacter* sp. by enzymes code by*MLr* homologous gene clusters should be known for the further study in future.

In the present study, biodegradation of MC was experimentally tested using native bacterial communities to perform MC-degrading was eliminate for 9 or 10 days. This time is matching point with that reported by Christoffersen *et al*. [24], who confirm the degradation of the two microcystin analogues in natural waters were almost removed around 8 days. However, some reports found that there is a lag-phrase ranging from 2 to 20 days in different surface waters and different initial concentrations of MC until the biological degradation commence [25].

V. CONCLUSIONS

In the present study, a native bacterium was isolated from Lake Taoranting which could be adapted to perform biodegradation of MC-LR and MC-RR. Base on 16S rDNA gene analysis, the strain was identified as *Arthrobacter* sp. Batch experiments using isolate T11 as the sole bacterium inoculums demonstrated effective degradation of MC-LR and MC-RR using sterilized Lake water with complete removal observed within 10 days. Therefore, such bacteria, intact with MC in natural water, maybe contribute to the self-purification of ecosystem from such potent toxins.

VI. ACKNOWLEDGEMENTS

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