Bacterial Degradation of Microcystins within a Biologically Active Sand Filter

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Abstract—A bacterium, Novosphingobium sp. KKU03, previously demonstrated to degrade the cyanobacterial toxin, microcystins (MCs), was investigated for the removal of MCs through the slow sand filter. In this study, biological sand filtration was assessed in PVC column experiment for its ability to remove MCs (MC-LR and MC-LR). Degradation of MCs was observed with inoculated (6x10^7 CFU/ml) treatment of water dosed with both MCs (completed degradation within 7 days) compared to uninoculated control. Polymerase chain reaction (PCR) specifically targeting amplification of 16S rRNA gene of Novosphingobium sp. KKU03 was applied to monitor the presence of the bacterium in experimental trials. PCR products indicative of an endemic bacterial population were observed at all sample sites through the column where MCs degradation was measured, indicating this bacterial isolate was active in degradation of MCs.

I. INTRODUCTION

Toxic cyanobacterial blooms are frequently observed in eutrophicated fresh water bodies throughout the world. Several genera are known to produce toxic secondary metabolites—cyanotoxins [1]. Microcystins (MCs) are the most commonly found and widespread cyanotoxins, being associated with several bloom-forming genera, especially Microcystis [2], [3]. Contamination of MCs in water bodies has led to fatalities in wild and domestic animals worldwide [4], and the toxins have also been associated with primary hepatocellular carcinoma in human [5], [6].

With their chemically stable cyclic heptapeptides structure, MCs are difficult to remove during traditional water treatment processes [7]. Therefore, many studies on removal of MCs from water bodies have been performed. Biological degradation of MCs in natural lakes and reservoirs has been reported [8], [9]. Only a few reports have related degradation of MCs in biologically active sand filters. Biological sand filtration systems are becoming more attractive to water suppliers as they are generally of low technology requiring little maintenance and infrastructure [10]. Biodegradation of MCs is mediated by the biological activity in the filters due to attachment and growth of biofilm microorganisms [11]. As MCs are readily degraded by aquatic microorganisms in natural water [12], MCs degradation could occur through sand filtration systems where microorganisms would exist within the biofilm of the filters.

The objective of this study was to determine the removal of MCs under slow sand filtration condition with the isolated bacterium Novosphingobium sp. KKU03. Molecular analysis based on the 16S rRNA gene sequence was undertaken to detect the MCs-degrading bacterium through the sand filter column.

II. MATERIALS AND METHODS

A. Source of MCs

Microcystis aeruginosa KKU-1 was isolated from Bueng Nong Khot, Khon Kaen, Thailand in 2010. The cells were grown in MLA medium [13] with bubbling air at room temperature under a light intensity of 20 μmol photons m⁻²s⁻¹, with a 12/12 h light/dark cycle. The cell suspension of 10 ml each were collected and their absorbances were read at 238 nm. Eluates were then pooled into fractions a-e.

B. Extraction and Separation of MCs

MCs were extracted from the lyophilized cells and separated by DEAE anion exchange chromatography according to the method described by Saito et al. [14]. The fractions (10 ml each) were collected and their absorbances were read at 238 nm. Eluates were then pooled into fractions a-e.

C. Analysis of MCs with HPLC

MCs analyses were carried out by HPLC with UV-detection at a wavelength of 238 nm. The mobile phase was acetonitrile: 0.05M phosphate buffer pH 3.0 (30:70 v/v) at a flow rate of 1 ml/min. The HPLC system consisting of a 600 pump controller, 717 plus autosampler, 2487 dual λ absorbance detector (Waters Ltd. USA.) and a TSK-GEL ODS-80Ts Column (150x4.6 mm) (Phenomenex, USA) was employed.

D. Degradation of MCs by the Bacterium

- Bacterial strain

A bacterium KKVU03 was isolated from Nong Kin Moo, Khon Kaen, Thailand in 2010 where a Microcystis bloom had been occurred. The taxonomic characterization was carried out using morphological observations, biochemical analyses, and 16S rRNA sequencing. The results indicated that the isolate KKVU03 was identified to Novosphingobium sp. Therefore, this isolate was named Novosphingobium sp. KKU03.
Degradation of MCs by Novosphingobium sp. KKU03

Degradation of MCs by Novosphingobium sp. KKU03 was investigated according to the method described by Jones et al. [15]. Briefly, the bacterial cell were harvested, washed, and transferred to conical flasks containing MSM. MCs fraction c (Dha\(^7\) MC-LR and MC-LR) from DEAE column were added to give final concentration of 5 \(\mu\)g/ml. Sample were incubated at 30 °C on an orbital shaker at 150 rpm. MCs degradation was monitored by centrifugation of an aliquot (1 ml) for 10 min at room temperature to remove the cell. The supernatants were assayed for remaining MCs.

Degradation of MCs using sand filtration

This experiment was performed using a biologically active slow sand filter according to the method described by Bourne et al. [16]. Sand filters were washed with MSM through the columns for 24 h prior to inoculation. The first sand filter was inoculated with Novosphingobium sp. KKU03 (6x10\(^7\) CFU/ml, 20 ml) by injection through the inoculation port above the sand bed. The second sand filter was left uninoculated. After addition of cells to the top of the sand bed, an adhesion period for 1 h was allowed before MCs ([Dha\(^7\)MC-LR and MC-LR) were added at a final concentration of 5 \(\mu\)g/ml to fill the columns. The peristaltic pump was turned on at a flow rate of 0.5 ml/min (the time defined as T=0), and experimental sampling of the columns commenced. The concentration of the MCs in water eluting from the columns were measured by HPLC daily.

Sand cores (1 g) were removed from the sampling ports for DNA extraction and PCR amplification. Cores were removed from sampling port 1 at T=0 and 24 h, and from all ports down the column (1-8) at T=14 days. DNA was extracted from sand samples using the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, USA) according to the manufacturer’s instruction. Partial sequence of the 16S rRNA gene was amplified and sequenced using primer pair 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3') and 16R1541 (5'-AAGGAGGT GATCCAGCCGCA-3'). The reaction mixture (50 \(\mu\)L) contained 20 ng genomic DNA, 0.5 \(\mu\)M of each primer (Invitrogen, Singapore), 1x Go Taq® Green Master Mix (Promega, USA), and 1 mM MgCl\(_2\) (Promega, USA). Thermal cycling was performed using a PTC-200 Peltier Thermal Cycler (MJ Research Inc., USA). The initial denaturation step at 94 °C for 2 min was followed by 30 cycles of DNA denaturation at 94 °C for 10 s, primer annealing at 55 °C for 20 s, and strand extension at 72 °C for 1 min. After cycling, a final extension step was performed at 72 °C for 7 min. The automated sequencing of the partial 16S rRNA gene was performed using the primer pair described above using the MegaBACE 1000 sequencing system (Amersham Biosciences, USA). Similar sequences to this 16S rRNA gene were searched for in the database of GenBank using a BLAST network service (BLASTN). Denomination of the bacterium was determined according to bacterial species having a similar identity with this 16S rRNA gene.

III. RESULTS AND DISCUSSION

Methods of MCs extraction and separation from cyanobacterial cells have been developed [14]-[17]. In this study, the toxins were extracted and then applied to DEAE column and eluted with linear gradient of NaCl. The chromatogram showed five major fractions. All fractions were identified by analytical HPLC and the results are shown in Table I. Based on the %yield of MCs, fraction c was obtained with the highest yield (87.83%). This fraction was composed of [Dha\(^7\)]MC-LR and MC-LR with 93 and 7% purity, respectively. Therefore, the fraction c was used for degradation experiment.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (mg)</th>
<th>Purity (%)</th>
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<tbody>
<tr>
<td>a</td>
<td>2.97</td>
<td>95% MC-RR and 5% MC-dMe-RR</td>
</tr>
<tr>
<td>b</td>
<td>0.01</td>
<td>94% MC-YR</td>
</tr>
<tr>
<td>c</td>
<td>51.84</td>
<td>93% [Dha(^7)]MC-LR and 7% MC-LR</td>
</tr>
<tr>
<td>d</td>
<td>3.16</td>
<td>94% MC-RR</td>
</tr>
<tr>
<td>e</td>
<td>0.04</td>
<td>92% MC-AR</td>
</tr>
</tbody>
</table>

Degradation of [Dha\(^7\)] MC-LR and MC-LR in the culture of Novosphingobium sp. KKU03 was observed followed analysis of the remaining MCs (Fig. 1a). There was a sharp decline during the first day of incubation and 92% of the toxins were eliminated in this period. Because the toxins could not be detected in the culture after 3 days of incubation, complete degradation was concluded.

Recently, several bacterial strains have been isolated and characterized with regard to their microcystin-degrading activities [18]-[20]. According to the study of Jiang et al. [7], Novosphingobium sp. THN1 was isolated from a water sample of Lake Taihu, China, which possessed complete degradation of MC-LR (1.38 \(\mu\)g/ml) after 60 h of incubation. In this study, the complete degradation of [Dha\(^7\)] MC-LR and MC-LR at higher concentration (5 \(\mu\)g/ml) was observed after 72 h of incubation.

The potential of using Novosphingobium sp. KKU03 capable of degrading MCs has been investigated using slow sand filtration column. In the study of biological active sand filtration column, complete removal of [Dha\(^7\)] MC-LR and MC-LR was observed within 7 days (Fig. 1b). Sand samples from port 1 at T=0 and T=24 h resulted in strong PCR detection of 16S rRNA gene of the bacterium. After 14 days of continuous passage of the toxins, sand removed from each port spaced down the column demonstrated positive 16S rRNA amplified signal for all samples, indicating the success of Novosphingobium sp. KKU03 to survive within the column.
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REFERENCES


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