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Elucidation of Microcystin-LR Degrading Mechanism of *Bacillus cereus*

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Abstract

Microcystin-LR (MC-LR) is a cyclic peptide produced as a secondary metabolite of certain freshwater cyanobacteria species such as, *Microcystis*, *Anabaena*, and *Oscillatoria*. Biodegradation of MC-LR by heterotrophic bacteria have been accepted as a reliable and cost effective method to treat MC-LR contamination. To date, more than 30 different bacterial genera have been recorded as potential MCs degraders. However, limited information are available on MC-LR degradation mechanism of bacteria. Intracellular MC-LR degradation by enzymes encoded by mlr A,B,C and D genes is the only hypothesis accepted so far. Hence, the present study focused on the elucidation of MC-LR degradation mechanism of KJ954304 *Bacillus cereus* 12GK strain which was previously isolated and characterized by authors as an efficient MC-LR degrader. Overnight grown and starved *B. cereus* bacterial suspension (0.5 µl) was inoculated into 100 ml of filter sterile (0.2 µm) Beira lake water containing MC-LR at a final concentration of 5 μgml^{-1}. Control sample was prepared without bacterial inoculation. All flasks were incubated at 28°C and shaken at 100 rpm for 3 days. Following three days of incubation 0.5 ml sub sample aliquot was removed from both experimental and control flasks and frozen at (-20°C). Then 45 ml of experimental sample was kept aside as initial experimental sample and remaining 45 ml of sample was filtered under sterile conditions using 0.2 µm filter to remove bacterial cells. Then the filtrate was placed immediately in ice to prevent denature of enzymes. Subsequently, MC-LR was spiked to both initial experimental samples and filtered samples at a final concentration of 5µgml-1, and incubated at 28^0C, 100 rpm for 3 days. 1ml aliquots were removed from initial experimental sample and filtrate sample for 0-3 days of incubation. These samples were frozen and processed for High Performance Liquid Chromatography (HPLC) analysis. A PCR analysis was carried out to detect the presence of MC-LR degrading mlr A,B,C and D genes in *B. cereus*. Amplifications were performed in µL volumes, containing 1 mM of each primer. A GeneAmps 2400 PCR System was utilized for the amplifications.

At the end of 3 days of experiment MC-LR concentration of the initial experimental sample with bacteria was 1.8 μgml^{-1} whereas the filtrate sample without bacteria had 4.8 μgml^{-1} of MC-LR. Furthermore, the PCR study confirmed the presence of mlr A,B,C and D genes in *B. cereus*. Thus, MC-LR degradation is performed as an intracellular degradation by *B. cereus* with the involvement of intracellular enzymes.

Keywords: Microcystin-LR, Biodegradation, *Bacillus cereus*, Intracellular degradation, mlr genes