BIODEGRADATION OF CYANOBACTERIAL HEPATOTOXINS [Dha$^7$]MC-LR AND MC-LR BY NATURAL AQUATIC BACTERIA

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Abstract

The aims of this doctoral study were to: isolate naturally occurring bacteria, able to degrade microcysts (MCs), from New Zealand waterbodies; to understand the biological processes of microcystin degradation by bacteria; and to develop small scale biofilm technology for testing the effectiveness of bacteria for microcystin degradation and/or remediation.

A significant amount of microcysts were required for biodegradation experiments. A modified method, using DEAE and Strata-X cartridge chromatography, was optimized for purifying microcystin variants from lyophilized bloom samples of the cyanobacterium Microcystis aeruginosa, collected en masse from Lake Horowhenua. Seven microcystin variants, MC-RR, MC-dMe-RR, MC-YR, MC-LR, [Dha\(^7\)]MC-LR, MC-FR, and MC-AR were purified by chromatography and then identified by reverse-phase High Performance Liquid Chromatography (HPLC) with UV detector (UVD) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). A mixture of [Dha\(^7\)]MC-LR and MC-LR, the main microcystin variants present, was used for examining biodegradation of microcysts by degrading bacteria.

Three isolates of bacteria—NV-1, NV-2 and NV-3—purified from Lake Rotoiti, New Zealand were capable of degrading [Dha\(^7\)]MC-LR and MC-LR. Among these isolates, NV-3 demonstrated the strongest degradative activity and was identified as a member of the genus Sphingomonas. On the basis of 16S rRNA sequencing, and 100% nucleotide sequence homology, it aligned most closely to strain MD-1. Based on the detection of two intermediate by-products (linearized peptides and a tetrapeptide) and the identification of four genes (mlrA, mlrB, mlrC and mlrD), that encode four putative proteins (enzymes) involved in microcystin degradation, it was suggested that the degradation of [Dha\(^7\)]MC-LR and MC-LR by the Sphingomonas isolate NV-3 occurred by a similar mechanism previously described for Sphingomonas strain MJ-PV (ACM-3962).
The bacterium *Sphingomonas* isolate NV-3 was examined for its ability to inhibit the growth of the cyanobacterium *Microcystis aeruginosa* strain SWCYNO4. It was found that the bacterium did not have any significant affect on the growth of the cyanobacterium, either by means of secretion of bacterial extracellular products or cell-to-cell contact between bacterial and cyanobacterial cells.

It was established that *Sphingomonas* isolate NV-3 was a moderate biofilm former, based on two types of biofilm formation assays, namely, microtiter plate assays and coupon biofilm assays. This was carried out in preparation for using the bacterium in a bioreactor for biodegradation of [Dha\(^7\)]MC-LR and MC-LR. The bacterium attached most effectively to ceramic, followed by PVC, polystyrene, stainless steel, and finally glass coupons. Biodegradation of MCs by the bacterium, in an internal airlift loop ceramic honeycomb support bioreactor (IAL-CHS bioreactor), was established in batch and continuous-flow experiments. In the batch experiment, NV-3 degraded a combination of [Dha\(^7\)]MC-LR and MC-LR at an initial concentration of 25 µg/ml at 30\(^\circ\)C in 30 hours, whereas in the continuous-flow experiment, NV-3 degraded the same concentration of [Dha\(^7\)]MC-LR and MC-LR in 36 hours with an hydraulic retention time (HRT) of 8 hours.

This study has demonstrated that microcystin-degrading bacteria are present in New Zealand waterbodies and that these bacteria could be used, potentially on a larger scale, for removing microcystins from water.
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