409/D

Application of molecular techniques for the detection of potentially microcystin-producing cyanobacteria in Kondawatuwana reservoir in Ampara

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The microcystins are a group of cyclic heptapeptide hepatotoxins produced by number of strains of cyanobacterial genera. The aim of this study was to determine the feasibility of using molecular probes to easily and accurately detect the potential for microcystin production by targeting the *mcyA* gene in microcystin biosynthetic (*mcy*) gene cluster. *mcyA* is responsible for the activation and incorporation of N-methyl-dehydro-alanin and L-alanin into the cyclic peptide which is essential for microcystin production.

Kondawatuwana tank (7° 17' 3" N 81° 38' 45" E) is a major irrigation tank located in the city of Ampara. The Ampara water supply scheme extracts raw water from Kondawatuwana reservoir and uses the Dissolved Air Flotation (DAF) technique to remove algae and other contaminants from water prior to filtration. Despite this treatment, the treated water from this water supply scheme turns dark brown from time to time. Predicting toxic blooms is important in view of both their increasing occurrence in the reservoir and the high cost of the current technology used for their removal.

Water samples were collected, inoculated into BG11 medium and incubated at 28+/- 2 ^{0}C with fluorescent light at a 16:8-h D/L cycle. Microscopic analysis was performed to investigate cyanobacterial composition and *Microcystis*-like species were tentatively identified from environmental samples. However, *Chroococcidiopsis* species was dominant in cultured isolates. Molecular analysis of the 16S rRNA region was used to detect cyanobacteria in water samples and cultured isolates. The 16S rRNA gene sequences confirmed that *Chroococcidiopsis* sp. AP2 (GU300772) was present in the cultured sample with 99% sequence similarity to *Chroococcidiopsis* sp. clone 1P-2-N12 (EU705152).

The presence and identification of toxic strains was studied by PCR amplification of *mcyA* gene in the microcystin synthesis pathway. Oligonucleotide primers (McyAF19/ McyAR47) were designed for the *mcyA* gene region. The presence of the *mcyA* gene involved in microcystin biosynthesis was found in one environmental sample (HQ848647), indicating the potential of this gene for producing the toxin. This PCR-based method could be a valuable tool for early detection of potentially toxic cyanobacteria in public water supply reservoirs in Sri Lanka before the appearance of cyanobacterial bloom and detectable level of toxin concentrations.

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