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### **Detection and quantification of microcystin in cultures of order Oscillatoriales cyanobacteria**

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Order Oscillatoriales cyanobacteria are considered to be an important group of harmful cyanobacteria as its members are found to be producers of microcystins (MCs). In Sri Lanka, order Oscillatoriales cyanobacteria are frequently recorded from freshwater reservoirs. Therefore, Oscillatoriales cyanobacteria can be considered as a potential risk for human health. Thus, the aim of this study was to detect potential toxicity of order Oscillatoriales cyanobacteria isolated from different parts of the country using PCR analysis, bioassay and protein phosphatase inhibition (PPI) assay.

A total of eight cyanobacterial mono cultures, belonging to the order Oscillatoriales by previous sequencing and identification (Mahapellassa hot spring; H1, H2, H5, tsunami affected areas; MI2, J4, D3a, YRS3 and Karavilahena wewa; DPW4) were used for the present study. Sub cultures were inoculated into BG11 medium and incubated at 28 +/- 2 °C with fluorescent light with intensity of 5.5 x 10<sup>-4</sup> cm<sup>-2</sup>W at a 16:8- h D/L cycle. The presence and identification of toxic isolates was studied by PCR amplification of *mcyE* gene in the microcystin synthesis pathway. A PCR fragment of ~ 480 bp was observed in H5, D3a and for *Leptolyngbya* DPW4 a fragment of ~ 450 bp was obtained. Other isolates, H1, H2, MI2, J4, and YRS3 gave null amplification for the *mcyE* gene.

Bioassay was performed using *Artemia salina* to test the toxicity of isolates. Results were analyzed with StatPlus 2009 Probit Analysis software and toxicity was expressed as LD<sub>50</sub> value. The highest toxicity was recorded in YRS3 isolate (LD<sub>50</sub>: -1,361.16 ppm), while H1, MI2, J4, D3a isolates showed -440.46, 3923.42, 734.80, 1703.49 ppm respectively and H2, H5, DPW4 isolates showed LD<sub>50</sub> value of -400.32 ppm. These findings showed that extracts of cyanobacterial isolates were toxic to *A. salina* from -1,361.16 ppm to 3923.42 ppm. The MicroCystest kit (ZEU-INMUNOTEC, S.L. Spain) was used to determine the intracellular MCs in the cultured isolates. The intracellular MC concentrations of H1, H2, H5, DPW4, MI2, J4, D3a, YRS 3 were found to be 0.38, 0.43, 0.49, 0.41, 0.54, 0.43, 0.52 and 0.44 µg/dm<sup>3</sup> respectively.

In conclusion, molecular data, bioassay and PPI assays have confirmed the presence of microcystin in all tested cyanobacterial isolates.

Keywords: bioassay, *mcy* gene, MicroCystest, microcystin, order Oscillatoriales