

Identification of cyanobacteria using 16S rRNA genes – A preliminary study

D T Gnanakkan, D N Magana – Arachchi* and P Jeyananadarajah

Institute of Fundamental Studies, Hantana Road, Kandy

The sequence analysis of genes encoding small subunit ribosomal RNA (16S rRNA) is currently the most promising approach for the phylogenetic classification of cyanobacteria. The aim of this study is to exploit molecular tools for characterizing and identifying cyanobacteria in their natural settings and phylogenetic classification of cyanobacteria based on the 16S rRNA genes. In this preliminary study, water samples from three different sites of the Kandy Lake were collected, inoculated onto appropriate media and were incubated at room temperature providing light for 16 hours. Samples were observed microscopically and the isolated microorganisms were tentatively identified to their genus level based on morphology: *Lyngbya*, *Merismopedia*, *Microcystis* and *Schizothrix*. DNA extractions were performed using standard methods. Lysis of bacterial cells was done by three sequences of freezing and thawing, followed by lysozyme and proteinase K treatment. Nucleic acids were purified by Boom's method using diatomaceous earth and guanidium isothiocyanate. DNA amplification was performed with cyanobacterial specific primers, forward primer Cya359F and a mixture of reverse primers Cya781Ra and

Cya781Rb. The assay was optimised with DNA extracted from a pure culture isolated from BG11 medium, in relation to the concentration of deoxyribonucleotides (dNTP), primers, *Taq* polymerase with 35 cycles. In this assay the expected fragment of 448 bp (16S rRNA gene) was amplified and detected by agarose gel electrophoresis. The filamentous form (TP 27) whose morphology could not be identified with known keys was found to be a cyanobacterium by PCR. A PCR product was also detected from the concentrated pellet of the water sample (X1). These preliminary results confirm that “DNA amplification of 16S rRNA gene” may be a useful tool in identifying cyanobacterial species in environmental samples.