

PERMANENT REFERENCE

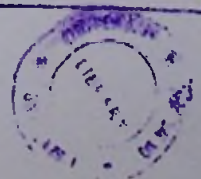


Identification and characterization of cyanobacteria in different climatic zones of Sri Lanka using 16S rRNA genes and detection of toxin producing cyanobacterial species using molecular markers

A thesis submitted to the Faculty of Science,
University of Colombo
for the Degree of Doctor of Philosophy

R.P. Wanigatunge
July 2014

PERMANENT
REFERENCE
NOT TO BE
REMOVED
FROM
THE



Abstract

Cyanobacteria, previously referred to as blue-green algae, are a diverse group of microorganisms that inhabit a wide range of ecological niches and are well known for their toxic secondary metabolite production. Knowledge of the evolution and dispersal of these microorganisms is still limited, and further research to understand such topics is vital. In Sri Lanka, a number of studies have been conducted for identifying fresh water cyanobacteria based on morphology, but molecular studies on cyanobacterial phylogeny and on cyanotoxin production have been scarce.

The objectives of the present study were to isolate and identify cyanobacteria using their morphological characters from different geographical locations of Sri Lanka, to determine the cyanobacterial phylogeny using 16S rRNA gene partial sequences, and to identify potential microcystin producing cyanobacteria from environmental samples and cultured isolates using molecular techniques. To determine the cyanobacterial diversity, samples were collected from water reservoirs representing wet, intermediate, dry and arid climatic zones. Sampling was also carried out from thermal springs, paddy fields, brackish water and tsunami affected areas (fresh water mixed with seawater).

Considering the distribution of cyanobacterial morphotypes, 37 genera were recorded from studied areas. Among them, 25 genera were observed in environmental samples representing the orders of Chroococcales, Pleurocapsales, Oscillatoriales and Nostocales. In cultured isolates, 35 genera were observed for all five cyanobacterial orders, including Stigonematales. *Scytonema* and *Anabaenopsis* species were only observed in environmental samples but could not get them into cultures. Cyanobacterial isolates belonging to genus *Gloeothece*, *Aphanocapsa*, *Planktolyngbya*, *Aphanizomenon*, *Tolypothrix*, *Hapalosiphon*, *Westiellopsis*, *Nodularia*, *Schizothrix*, *Cylindrospermum*, *Chlorogloeopsis* and *Fischerella* were only observed in cultures. According to the results obtained from present study, the highest diversity of cyanobacteria was recorded from dry zone reservoirs while the lowest cyanobacterial diversity was recorded from arid zone reservoirs. According to observations the genus *Planktolyngbya* and *Nodularia* were recorded from wet zone, genus *Anabaenopsis*, *Scytonema*, *Cylindrospermopsis*, *Tolypothrix*, *Aphanizomenon* and *Chlorogloeopsis* were recorded only from dry zone. In addition, genus *Coelosphaerium* was recorded only from intermediate zone. Microscopic examination of samples collected from hot spring showed a dominance of cyanobacterial species belonging to *Synechococcus*, *Chroococcus*,

Chroococidiopsis, *Xenococcus*, *Phormidium*, *Leptolyngbya*, *Calothrix* and *Chlorogloeopsis*.

In this study, the genus *Chroococidiopsis* was identified for the first time in Sri Lanka.

Ninety eight cyanobacterial isolates were identified using 16S rRNA sequences and were deposited in NCBI database. Eighteen isolates belonged to order Chroococcales, 49 to Oscillatoriales, ten to Pleurocapsales, six to Nostocales and three to Stigonematales. Order Oscillatoriales cyanobacteria were most abundant throughout the studied areas. Based on 16S rRNA gene sequences and their morphology, two isolates were identified up to species level, 72 to genus level, one isolate up to family and 11 up to order level. Twelve isolates could not be assigned to any taxonomic level. Three unclassified cyanobacteria did not show any sequence similarity to previously recorded sequences of known isolates recorded either from Sri Lanka or from other parts of the world. Therefore sequences of such species were deposited either as filamentous cyanobacterial species or unicellular cyanobacterial species according to their morphology. These cyanobacterial isolates could be considered as novel cyanobacterial genera. Nine other unclassified cyanobacterial isolates had sequence similarities ranging from 94%- 100% to previously recorded cyanobacteria, however considering their morphology or 16S rRNA gene sequences they could not be assigned to any taxonomic level.

According to the positions of the cyanobacteria in the phylogenetic tree, morphology could be correlated to the 16S rRNA sequences of unknown isolates. The 16S rRNA sequences of nine unclassified isolates were compared with known cyanobacterial sequences in GenBank and uncultured cyanobacterium Pa1 was identified to genus level as *Synechococcus*, uncultured cyanobacterium PFB-A2 to genus *Leptolyngbya*, unicellular cyanobacterium N2a to order level as Chroococcales cyanobacterium. These results confirmed the usefulness of 16S rRNA gene as a valuable tool for identification of cyanobacteria up to order or genus level.

Determination of potential microcystin producing ability of cyanobacteria was carried out to both cultured isolates and environmental samples using *mcyA* and *mcyE* genes in the microcystin synthetase pathway. Results confirmed the genetic potential of microcystin production of cultured isolates and environmental water samples collected from water reservoirs using both primers for *mcyA* and *mcyE* genes. Based on PCR results and analysis of nucleotide sequences of *mcy* gene confirmed the utility of molecular markers for early detection of potential microcystin producers in both environmental and cultured isolates.