# Morphological and Molecular Identification of Cyanobacteria Isolated from Selected Paddy Fields at RRDI, Batalagoda

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## Abstract

At present, 99% of the rice cultivation in Sri Lanka depends on chemical fertilizers whereas 35% of the rice area receives organic fertilizers together with chemical fertilizers. However, heavy and continuous use of chemical fertilizers has created negative effects on soil environment, i.e. soil acidity and salinity, degradation of soil structure, eutrophication in water bodies, destruction of biota and accumulation of harmful toxic chemicals as heavy metals. Latter have been related to major health issues of farming communities in rice growing areas e.g. Chronic Kidney Disease with Unknown etiology (CKDu) which is renamed as *Chronic Agrochemical Nephropathy*. Thus alternative strategies have been identified for improvement of soil fertility. Use of biofertilizersor microbial inoculants which facilitate transformation of unavailable forms of plant nutrients to available forms mainly via N fixation and P solubilisation is considered as one of the key alternatives for chemical fertilization in paddy cultivation.

Cyanobacteria are a morpho-physiologically diverse group of photoautotrophs which are found as a major group of micro biota dwelling in paddy fields, especially in the upper layer of soil and flood water. These have found to support crop growth by enhancing soil fertility directly via N-fixation, P solubilisation and secreting growth promoting substances and indirectly via improving soil structure and water-holding capacity. Therefore use of cyanobacterial bioertilizers are well adapted technology in other countries e.g. India. However, one of the issues of commercial formulations is less adaptation to local conditions after field inoculation. Use of inoculants which are isolated from local rice fields will solve this problem since these microbes are well acclimatized to the prevailing physicochemical and biological environment. The overarching goal of our study is to develop a successful biofertilizer for rice cultivation in Sri Lanka using cyanobacterial species isolated from local rice fields. In the current study we aimed for isolation and morphological and molecular identification of Cyanobacteria from selected rice fields at the Intermediate Zone of Sri Lanka.

Soil samples were collected from rice fields at the Rice Research and Development Institute (RRDI), located at Bathalagoda, (7°31'27.02"N, 80°26'57.08"E; 116 m above mean sea level) in the Intermediate Zone of Sri Lanka. Inorganic (ING) and Organic (ORG) treatment fields were selected for this study. ING fields were fertilized with urea, triple super phosphate, muriate of potash and $ZnSO_4$ according to the recommendation by the Department of Agriculture. ORG fields were amended with cow dung, compost and poultry litter at a rate of 3, 2.5, and 2 t/ha respectively. Fields were cultivated with BG 358 rice variety. The study site receives a mean annual precipitation of 1 100-1 600 mm and the soil is classified as low humicgley soils with clayey texture.

Fourteen cyanobacterial species/groups from paddy fields (0-10 cm depth) fertilized with inorganic or organic fertilizers were isolated and identified using culture based (BG11 medium) morphological analysis. Both unicellular (*Aphanothece, Chroococcus, Johannesbaptistia, Microcystis, Aphanocapsa, Synechococcus*) and filamentous (*Anabaena, Leptolyngbya, Pseudanabaena, Stigonema, Trichodesmium, Lyngbya, Oscillatoria, Nostoc*) genera of cyanobacteria were identified and axenic cultures were prepared.Molecular analysis confirmed the presence of both filamentous and unicellular cyanobacteria in studied paddy soils via DNA extraction using optimized protocols followed by PCR amplification using specific primers [CYA359F and CYA781R (a)] and [CYA359F and CYA781R (b)] respectively. Axenic cultures of two unicellular cyanobacterial genera (*Chroococcus* and *Microcystis*) were successfully confirmed using specific primers. Sequencing results showed 93% homology with the cyano bacterial sequence reported in NCBI Database (JF988255). Relative abundance of each identified genera (based on Colony Forming Units) showed no significant differences between ORG and ING samples. *Aphanothece* and *Synechococcus* were the highest abundant whereas *Leptolyngbya, Oscillatoria* and *Johannesbaptistia* were found in very low abundance in both fields.

**Keywords:** Biofertilizer; Paddy; Cyanobacteria; Specific primers

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