Biodiversity analysis of selected cyanobacteria

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Cyanobacterial strains of *Nostoc*, *Anabaena* and *Calothrix* were examined for pigments, carbohydrates, total soluble proteins and enzymes related to N-metabolism. Total chlorophyll content was highest in *Anabaena* followed by *Nostoc* and *Calothrix*. *Calothrix* exhibited highest carotenoid content followed by *Anabaena* and *Nostoc*. In *Anabaena*, allophycocyanin was highest followed by phycocerythrin and phycocyanin. In *Nostoc* and *Calothrix*, phycocerythrin was highest followed by allophycocyanin and phycocyanin. Carbohydrate content and total soluble proteins varied amongst various isolates and a notable difference was seen in nitrate reductase and glutamine synthetase (transferase) activity. Based upon the similarity matrix, a dendrogram was constructed using SIMQUAL software. Inter/ intrageneric similarity was recorded for physiological parameters when assigned equal importance, and some of the cyanobacterial isolates exhibited either no or only 6% similarity. All the clusters showed isolates from more than one genus, thus indicating that the cyanobacterial genera cannot be differentiated based upon physiological parameters alone.

**Keywords:** Analysis, biodiversity, cyanobacteria, physiological parameters.

**CyanoBacteria**, the oxygenic, photosynthetic prokaryotes are found in almost every aquatic and terrestrial environment. They show remarkable degree of morphological and developmental diversity. Traditionally, their taxonomy is based upon morphological and physiological observations. Taxonomy, on the basis of morphological characters, has been debated vigorously and revised many a times. In addition to morphological diversity and widespread distribution, cyanobacteria reflect a broad spectrum of physiological properties and tolerance to environmental stress. The most recent taxonomic revision based on morphological, biochemical and molecular characters is an extensive compendium by Anagnostidis and Komárek. Presently, *Bergey’s Manual* has placed BGA/cyanobacteria under phylum BX, Cyanobacteria (Oxygenic Photosynthetic Bacteria). Morphological and physiological parameters such as growth, composition of pigments, N-assimilatory enzymes and certain metabolites are considered important for differentiation of cyanobacterial strains. Appropriate combination of morphological attributes and physiological parameters can aid in clarifying phylogenetic relation-

In view of this, cyanobacterial isolates from three genera namely *Nostoc*, *Anabaena* and *Calothrix* were analysed (a) to study the inherent variation in terms of physiological/biochemical parameters, and (b) to examine inter- as well as intra-generic relatedness using statistical software package.

Cyanobacterial isolates *Nostoc* (Nsl to Ns15), *Anabaena* (An1 to An15) and *Calothrix* (Ca1 to Ca15) were procured from germplasm of CCUBGA, IARI, New Delhi for the present study. These were maintained in BG-11 medium under controlled illumination (4000 lux) with 16/8 h light/dark cycle at 28 ± 2°C. Identification was confirmed based upon the keys given by Desikachary for microscopic parameters. The isolates were examined for pigments, total soluble proteins, carbohydrates and N-assimilatory enzymes (nitrate reductase and glutamine

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Comparative photosynthetic pigments in cyanobacterial isolates of *Nostoc*, *Anabaena* and *Calothrix*.
synthetase activity) at exponential stage during growth. Dry weight was measured using hot-air oven at 60°C. Chlorophyll was estimated by hot methanol extraction method. Carotenoids were extracted in acetone and phycobiliproteins were determined in phosphate buffer. Nitrate reductase activity was studied by diazo coupling method and glutamine synthetase (transferase) activity calculated using standard curve of γ-glutamyl hydroxamate. Based upon the data, similarity coefficient was calculated on the principle of Jaccard for physiological parameters when assigned equal importance and the dendrogram made using SIMQUAL software.

Physiological diversity within cyanobacterial genera has been addressed in studies of cultural isolates and their response to light and nutrients. The present study has been carried out on 45 unicellular axenic isolates (15 each) from *Nostoc*, *Anabaena* and *Calothrix* respectively. Cyanobacterial genera are reported to have specific pigments like chlorophyll *a*, phycobiliproteins and carotenoids. Total mean chlorophyll was highest in *Anabaena* followed by *Nostoc* and *Calothrix*. *Calothrix* showed highest carotenoids followed by *Anabaena* and *Nostoc*. In *Anabaena*, allophycocyanin was highest followed by phycoerythrin and phycocyanin, while in *Nostoc* and *Calothrix*, phycoerythrin was highest followed by allophycocyanin and phycocyanin. In *Nostoc* isolates, chlorophyll content ranged from 13.8 to the lowest of 2.9 µg ml⁻¹, while in the isolates of *Anabaena*, chlorophyll content ranged from 3.4 to the highest of 11.4 µg ml⁻¹ and in *Calothrix* isolates, pigment content ranged from 4.3 to 8.6 µg ml⁻¹. Carotenoids ranged from 29.2 to 7.3 µg ml⁻¹, 18.6 to 5.9 µg ml⁻¹ and 21.4 to 6.5 µg ml⁻¹ in *Nostoc*,

![Figure 2](image-url) Comparative metabolites (total soluble proteins and carbohydrates) in cyanobacterial isolates of *Nostoc*, *Anabaena* and *Calothrix*.

![Figure 3](image-url) Comparative N-assimilatory enzymes (GS and NR) in cyanobacterial isolates of *Nostoc*, *Anabaena* and *Calothrix*.
Anabaena and Calothrix respectively. Variations in the pattern of carotenoid composition are reported to be useful for species identification in cyanobacteria. Similar carotenoid patterns were observed for Phormidium ectocarpri strain PCC7375 and Phormidium persicinum strain CCAP469. However, carotenoid content and composition were different in red or green isolates of the same species, suggesting the taxonomic utility of this pigment. Total phycobiliproteins varied from 63.9 to 15.7 μg ml⁻¹ and 93.9 to 13.0 μg ml⁻¹ in Nostoc and Anabaena respectively. Amongst Calothrix isolates, the total phycobiliproteins ranged from the highest value of 59.9 μg ml⁻¹ to the lowest of 28.2 μg ml⁻¹ (Figure 1). When phycobiliprotein pattern of 21 cyanobacterial species was investigated by polyacrylamide disc gel electrophoresis, a distinct heterogeneity was observed even between strains assigned to the same species.

Reviews on secondary metabolites produced by Lyngbya and other filamentous cyanobacteria stressed upon tremendous variations in the quantity and quality amongst collections. The total soluble proteins and carbohydrates were highest in Calothrix followed by Nostoc and Anabaena for proteins and Anabaena and Nostoc for carbohydrates (Figure 2). In Nostoc, the total soluble proteins varied from 176.5 to 39.9 mg ml⁻¹, while in Anabaena, it varied from the highest value of 245.9 to the lowest of 72.8 mg ml⁻¹. Amongst Calothrix isolates, total soluble proteins ranged from the highest 420.64 to the lowest level of 190.8 mg ml⁻¹. Different values for the average protein or N content and the number of heterocysts have been reported between species with varying growth conditions. Carbohydrates ranged from the highest 165.3 to the lowest of 48.1 mg ml⁻¹ amongst Nostoc isolates. In Calothrix, it ranged from 642.9 to the lowest of 290 mg ml⁻¹ (Figure 2).

There was a significant difference amongst the isolates of three cyanobacterial genera with respect to the enzymes nitrate reductase (NR, EC 1.6.6.1) and glutamine synthetase (GS, EC 6.3.1.2; Figure 3). NR enzyme activity was highest in Anabaena followed by Nostoc and Calothrix. On the contrary, Anabaena showed highest GS activity followed by Nostoc and Calothrix. Nitrate reductase activity ranged from 58.7 to 7.1 μg NO₃⁻ ml⁻¹ in Nostoc while in Anabaena, it varied from 140.6 to 16.8 μg NO₃⁻ ml⁻¹. However, in Calothrix, the highest NR activity (35.4 μg NO₃⁻ ml⁻¹) was followed by the lowest of 15.5 μg NO₃⁻ ml⁻¹. Glutamine synthetase (GS) activity (nmol γ glutamyl hydroxamate μg⁻¹ protein, 30 min⁻¹) ranged from 15.6 to 36.2 in Anabaena and amongst Nostoc strains, it ranged from the highest value of 22.6 to the lowest of 11.4. In Calothrix, enzyme activity ranged from 17 to 34 nmol γ glutamyl hydroxamate μg⁻¹ protein, 30 min⁻¹ (Figure 3).

The results in the dendrogram depicted interfirna generic similarity in terms of physiological parameters when assigned equal importance. Some of the cyanobacterial isolates exhibited either no or only 6% similarity. There were
two clusters having 22% similarity, each with five and forty isolates respectively. The cluster having forty isolates could be further divided into two subclusters with 2 and 38 isolates respectively. These two subclusters exhibited 28% similarity. The cluster with 38 isolates could be further differentiated into three clusters, each having 6, 5 and 27 isolates with about 40% similarity. All the clusters showed isolates from more than one genus with a few exceptions (Figures 4 and 5). Hence, the genera could not be differentiated based upon physiological parameters alone. The results clearly exhibited diversity among cyanobacterial isolates with respect to physiological parameters traditionally assigned to *Nostoc*, *Anabaena* and *Calothrix*.

Cyanobacteria are important and potential genetic resources due to immense agricultural and biotechnological implications. Hence, an appropriate combination of morpho/physiological parameters should aid in clarifying phylogenetic relationships. Distinct variations in growth attributes and other physiological parameters were observed. The dendrogram depicted that the cyanobacterial genera cannot be differentiated based upon physiological parameters alone.

The investigation clearly revealed and highlighted the need for a morpho-physiological and molecular approach for cyanobacterial characterization and their utilization in agriculture and industry.

Cloning partial endochitinase cDNA of *Trichoderma harzianum* antagonistic to *Colletotrichum falcum* causing red rot of sugarcane

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The saprophytic fungus, *Trichoderma harzianum* strain T5 antagonistic to the red rot pathogen of sugarcane, *Colletotrichum falcum* excreted chitinases, viz. N-acetyl-β-d-glucosaminidase, 1,4-β-d-N-N' chitobiosidase and 1,4-β-d-N-N'-N' chitotriase into the culture medium containing pathogenic cell wall or chitin. The protein profile analysis on SDS–PAGE stained proteins is in the range of 20–124 kDa. The fungus produced a 97 kDa N-acetyl glucosaminidase in medium amended with fungal cell wall or colloidal chitin. Chitobiose isoforms of 66, 56, and 50 kDa and 66 and 50 kDa were detected in fungal cell wall and colloidal chitin amended media respectively. The fungus also excreted chitotriase isoforms of 66 and 50 kDa in cell wall and 50 kDa in colloidal chitin amended media respectively. The chitinase enzymes of 66 and 50 kDa, which degraded both chitobiose and chitotriose, are isoforms of both chitobiose and chitotriose that got separated at the same distance in SDS–PAGE. In an attempt to clone the endochitinase gene from *T. harzianum* T5, a partial cDNA of 246 bp (Genbank accession number AM72230) was obtained through RT–PCR and the deduced sequence showed high level of homology with chitinase sequences of the database.

**Keywords:** cDNA cloning, chitinases, endochitinase, *Trichoderma harzianum*.

Chitin is a significant component in the cell walls of large groups of fungi except members of Oomycetes. Chitin is made up of molecules of N-acetylglucosamine, which are the building blocks linked together by 1,4-β-glycosidic bonds. Chitinases are enzymes that cleave the bond between the C1 and C4 of two consecutive N-acetylglucosamines of chitin. Chitinases produced by fungi have been shown to be involved in a variety of functions such as cell-wall digestion, germination of spores, assimilation of chitin and mycoparasitism. Thus chitinases have the potential as effective antifungal agents¹. The chitinolytic system of *Trichoderma* spp. is much diversified. It is obvious that homologues of a 42 kDa endochitinase³ have often been purified⁴, followed by frequent isolation of an N-acetyl-α-d-glucosamine of 70–73 kDa. The gene encoding the 42 kDa endochitinase has high homology to endochitinases from other fungal species⁵ and hence is a useful gene for phylogenetic analysis of *Trichoderma, T. harzianum* strain T5 isolated from sugarcane rhizosphere displayed antifungal activities against *Colletotrichum falcum* Went (perfect state: *Glomerella cucumerina* (Speg.) Arx & Muller), causing red rot in sugarcane⁶. Mycoparasitic fungi provide rich sources of antifungal genes that can be utilized to genetically engineer important crops for resistance against fungal pathogens. We report here detection of three extracellular chitinases of *T. harzianum* produced on C. falcum cell wall and colloidal chitin amended media and cloning its partial endochitinase gene sequence.

* T. harzianum strain T5 that has been isolated from sugarcane rhizosphere was grown in a minimal medium containing KH₂PO₄ – 1.5 g; MgSO₄·7H₂O – 1.5 g; and cell-wall chitin or colloidal chitin – 1 g/l. Colloidal chitin was prepared by digesting powdered crab shells (Sigma Aldrich, USA) with concentrated HCl overnight at 4°C. Cell-wall chitin was prepared from mycelial mats of *C. falcum* as described earlier⁷, in which mycelia from eight-day-old cultures were homogenized in a homogenizer for 60 s using 5 ml water/g wet weight of mycelia. The homogenate was

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