Molecular characterization of selected cyanobacterial strains

8.1 Introduction

Molecular (phylogenetic) tools have now been widely applied (Robertson *et al.*, 2001) which provide a basic criterion for taxonomic classification (Komárek, 2006) and seem to be very important for taxonomy and further phylogenetic investigations, revealing

necessity of separation of polyphyletic taxa into a number of narrower monophyletic genera or cryptogenera (Korelusová, 2008). Thus isolates should be studied by combining morphological, chemotaxonomic and genetic approaches (Rajaniemi *et al.*, 2005; Prakash *et al.*, 2007) which leads to the term "polyphasic approach" of classification system which was first mentioned by Colwell (1970). The specific marker like 16S rDNA, phycocyanin locus, nif gene, rpo gene, ITS region, phosphoenolpyruvate carboxylase gene etc (Smith et al., 2008) are being used for studying the molecular assessment of cyanobacteria biodiversity. The application of denaturing gradient gel electrophoresis (DGGE) along with PCR for studying natural cyanobacterial assemblages has increased our understanding of their complexity in environmental samples (Muyzer et al., 1993). The studies of genetic relationships among non-axenic cyanobacteria and without cultivation of strains were made possible due to the application of molecular biological methods (Gurtler and Mayall 2001) and cyanobacterial-specific primers (Urbach et al., 1992).

8.2 Methodology

In detail of the methodology is already mentioned in **chapter 3**. In brief, Genomic DNA from the isolated cyanobacteria was extracted by standard procedure (Smoker and Barnum, 1988). 16S rRNA gene was amplified with the following primers:

CYA 106f (5′- CGGACGGGTGAGTAACGCGTGA -3′) and

CYA781 r (a) (5′GACTACTGGGGTATCTAATCCCATT-3′) (Nübel *et al.* 1997).

Thermo-cycling condition for the PCR was followed as initial denaturation at 94°C for 5min; 30cycles of 94°C for 1min (denaturation), 58°C for 45sec (annealing), 72°C for 1min (elongation) and final elongation at 72°C for 7min. Sequencing was done with

respective forward and reverse primers with the amplified sample and the obtained sequence was checked for the homology to other sequences deposited in the available databases using Basic Local Alignment Search Tool (BLAST) search (http://www.ncbi.nim.nih.gov/BLAST). The gene sequence was submitted to Genbank under the respective accession numbers. Phylogenetic analysis of the DNA sequence data was performed with MEGA5 software (Tamura *et al.*, 2005).

8.3 Results and Discussion

8.3.1 Phylogenetic analysis

Among the various gene sequences used to assess cyanobacterial biodiversity, 16S rRNA gene has been applied most frequently (Robertson et al., 2001). variation. A total of 8 cyanobacterial strains were screened for the molecular characterization **(Table 7.1)**. Phylogenetic tree was constructed with the sequences which are available in public database of NCBI. The amplified products of 16S rDNA of approximately 750bp size **(Fig. 7.2)**. Nucleotide sequences obtained for the strains were compared with already available sequences in the NCBI (National Center for Biotechnology Information) database using BLAST (Basic Local Alignment Search Tool). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.98831349 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site.

The analysis involved 23 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 424 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. In the tree **(Fig.7.3)** closely related strains were clustered together. A distinct clade between heterocystous and non heterocystous cyanobacteria was observed in the tree. The taxa of the order Nostocales and Oscillatoriales were found to be polyphyletic forming distinct and separate clusters.

7.3.2 Development of cyanobacterial germplasm

All the 27 cyanobacterial isolates **(Table 7.2)** were purified and strains were maintained in agar slants **(Plate 7.1; a-d)** for the deveopment of cyanobacterial germpalsm. Strains were submitted to the algal repository **(Plate 7.2)** developed in the Department of Ecology and Enviromental Science, Assam University, Silchar.

7.4 Conclusion

A congruence between morphological and the phylogenetic tree of the isolated strains based on 16S rDNA analysis were found. Inspite of the congruence between morphometric and the 16S rRNA gene sequence the phylogenetic study of some strains were, however, found to be not quite consistent with the morphological classification. The study necessitates more isolation and characterization of cyanobacterial strains with the increase in its DNA database from this unexplored region.

Table8.1: Details of cyanobacteria and their % similarity using BLAST searches from gene sequences retrieved from NCBI GenBank

Sl no.	Name of the strain	% Similarity through BLAST
1	*Scytonema tolypothrichoides* AUS-JR/AP/NT-076	96% with *Scytonema* sp. (KC682102)
2	*Cylindrospermum muscicola* var. *longispora* AUS-JR/AP/NT-077	95% with *Cylindrospermum muscicola* (KM019946)
3	*Oscillatoria formosa* AUS-JR/AP/NT-078	95% with *Oscillatoria subbrevis* (KJ546666)
4	*Lyngbya polysiphoniae* AUS-JR/AP/NT-079	97% with *Lyngbya* sp. (KP178669)
5	*Phormidium angustissimum* AUS-JR/AP/NT-081	99% with *Phormidium articulatum* (KP297409)
6	*Lyngbya polysiphoniae* AUS-JR/AP/NT-082	97% with *Lyngbya* sp. (KP178669)
7	*Fischerella muscicola* AUS-JR/AP/NT-083	99% with *Fischerella muscicola* (AM709634)

The sequencing results of all the 7 cyanobacterial genera were analyzed using BLAST search in NCBI website. The two cyanobacteria *Cylindrospermum muscicola* var. *longispora* and *Fischerella muscicola* were identified morphologically and confirmed by sequencing data. The other 5 cyanobacteria *Scytonema* sp. *Oscillatoria subbrevis*,

Lyngbya sp., *Phormidium articulatum* and *Lyngbya* sp. were morphologically identified as *Scytonema tolypothrichoides, Oscillatoria formosa, Lyngbya polysiphoniae, Phormidium angustissimum* and *Lyngbya polysiphoniae*. Using the sequencing data these species were named accordingly with the strain name as per the morphological observation.

Table 8.2: Strains submitted to the NCBI GenBank

Sl no.	Name of the strain	Accession No.
1	*Scytonema tolypothrichoides* AUS-JR/AP/NT-076	KX670242
2	*Cylindrospermum muscicola* var. *longispora* AUS-JR/AP/NT-077	KX670243
3	*Oscillatoria formosa* AUS-JR/AP/NT-078	KX670244
4	*Lyngbya polysiphoniae* AUS-JR/AP/NT-079	KX670244
5	*Phormidium angustissimum* AUS-JR/AP/NT-081	KX670245
6	*Lyngbya polysiphoniae* AUS-JR/AP/NT-082	KX670246
7	*Fischerella muscicola* AUS-JR/AP/NT-083	KX670247

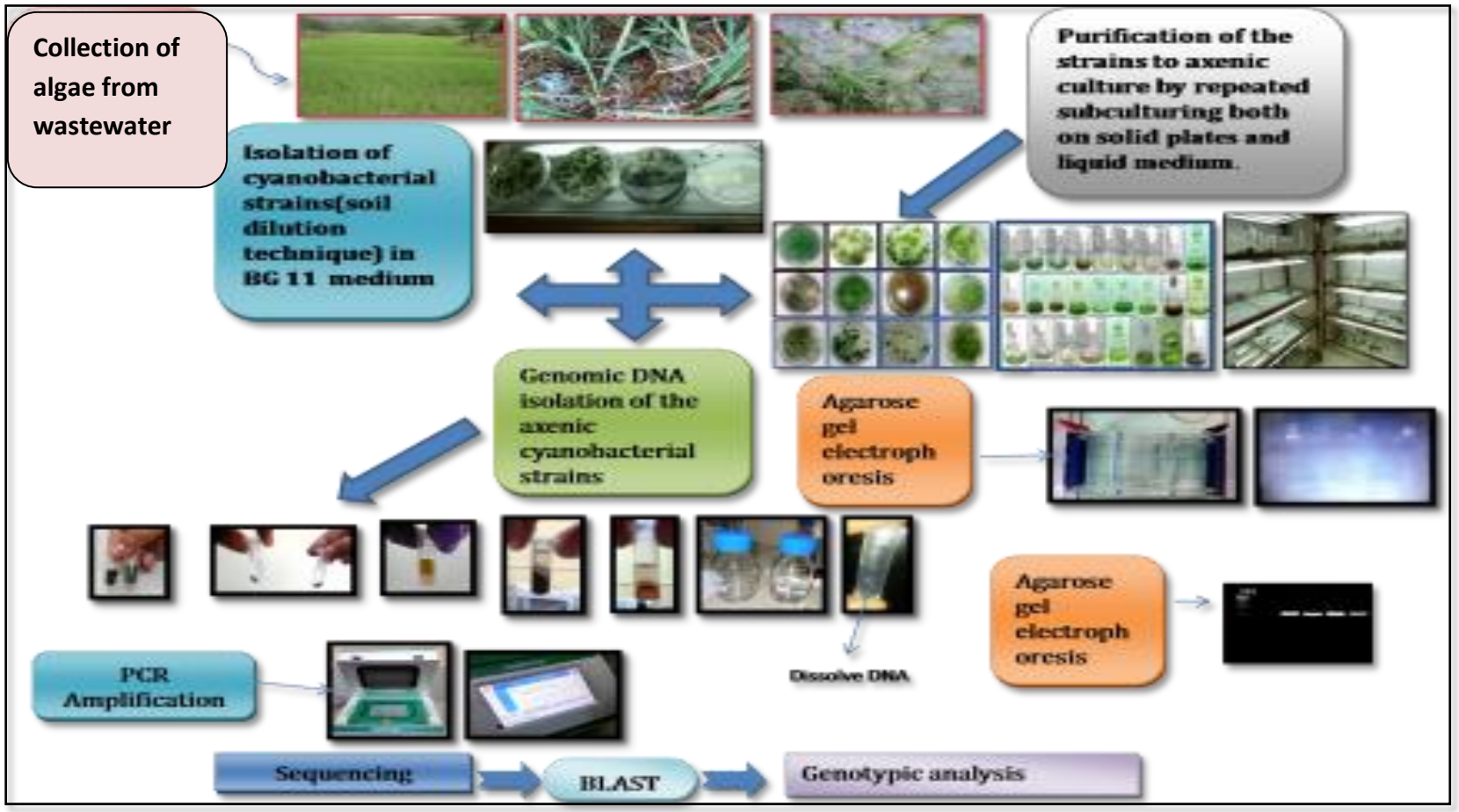

Plate: 8.1 Flow chart exhibiting the collection to isolation and molecular characterization of the algae

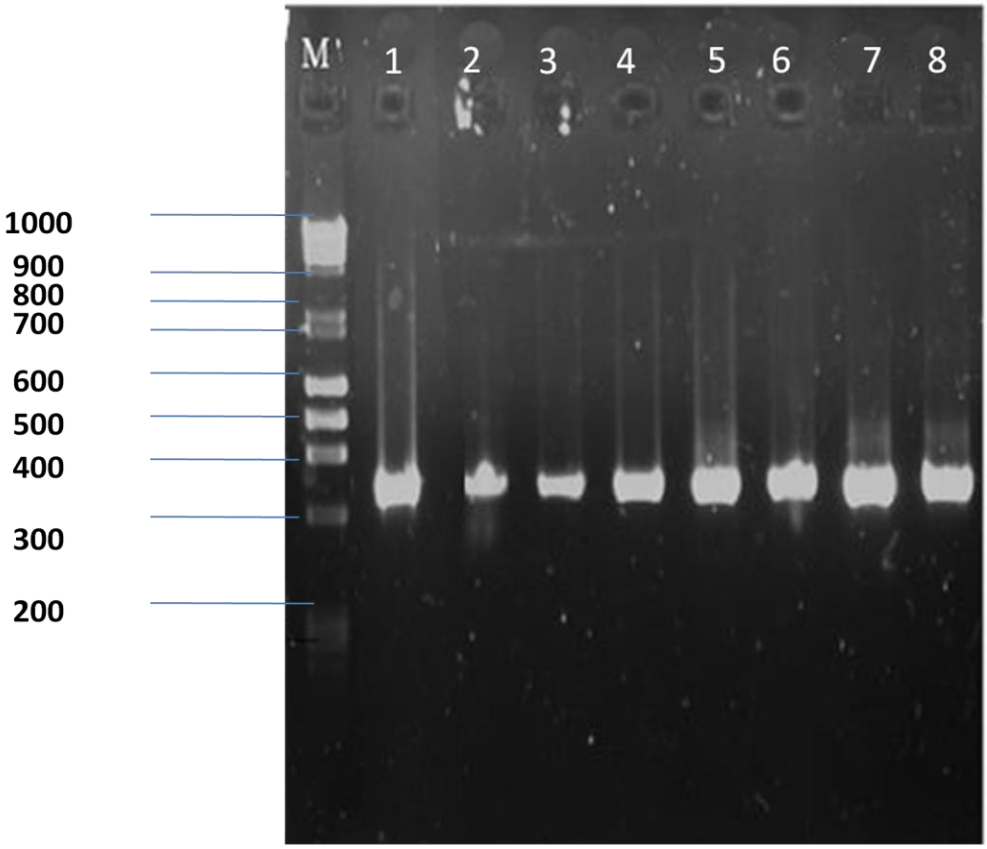

Fig.8.1: 16S rDNA amplification of selected cyanobacterial strains, 1) *Scytonema tolypothrichoides* 2) *Cylindrospermum muscicola* var. *longispora* 3) *Oscillatoria formosa* 4) *Lyngbya polysiphoniae* 5) *Nostoc linkia* 6) *Phormidium angustissimum* 7) *Lyngbya polysiphoniae* 8) *Fischerella muscicola*

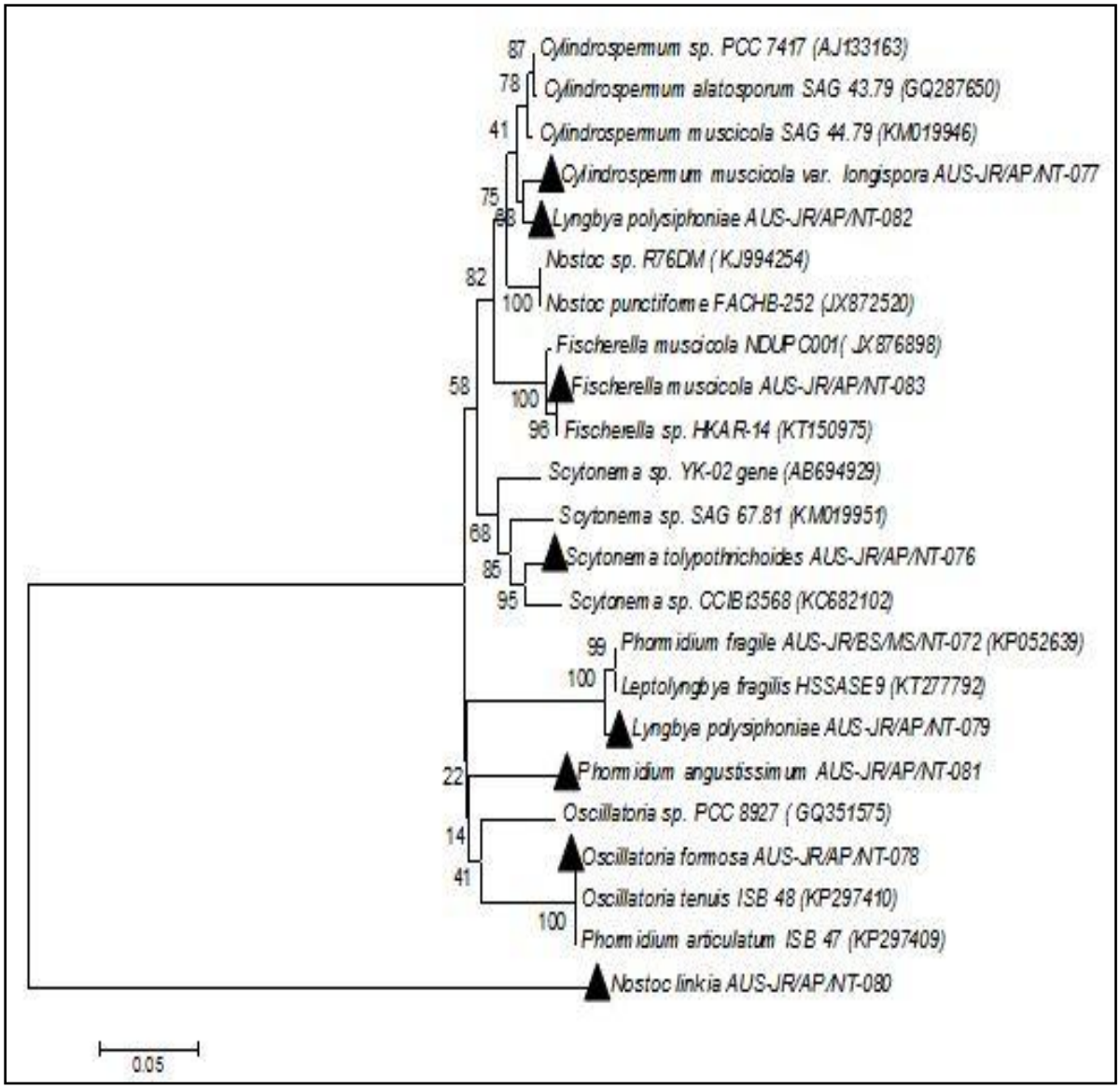

Fig. 8.2: The distance matrix tree of the strains isolated from the agro-ecosystems based on partial 16S rRNA sequence

Table 8.2: Cyanobacterial isolates submitted to the Algal repository developed in Department of Ecology and Environmental Science, Assam University, Silchar, Assam

Sl. no.	Name of the organism	Source of Isolation	Abbreviation used
1	*Anabaena doliolum* Bharadw. (after Bharadwaja)	Solid wastes deposits	CYA134
2	*Anabaena orientalis* Dixit (after Dixit)	Solid wastes deposits	CYA135
3	*Anabaena spiroides* var. crassa Lemmermann	Solid wastes deposits	CYA136
4	*Aphanothece bullosa*	Paper mill wastewater	CYA137
5	*Calothrix marchica* Lemm. (after Fremmy)	Paper mill wastewater	CYA138
6	*Calothrix marchica* v. *intermedia* Rao, C.B. (after Rao, C.B)	Solid wastes deposits	CYA139
7	*Calothrix marchica* v. *crassa* Rao, C. B. (after Rao, C. B.)	Solid wastes deposits	CYA140
8	*Cylindrospermum majus* Kutz. (after Frémy)	Paper mill wastewater	CYA141
9	*Cylindrospermum musicola* Kutzing ex. Born ex. Flah	Paper mill wastewater	CYA142
10	*Cylindrospermum muscicola* var. longispora Dixit	Paper mill wastewater	CYA143
11	*Fischerella muscicola* (Borzi) Gomont	Solid wastes deposits	CYA144
12	*Lyngbya polysiphoniae* Frémy (after Frémy)	Paper mill wastewater	CYA145
13	*Lyngbya limnetica* Lemmermann	Solid wastes deposits	CYA146
14	*Nodularia spumigena* Mertens in Jürgens	Paper mill wastewater	CYA147
15	*Nostoc carneum* Ag. (after Frémy)	Paper mill wastewater	CYA148
16	*Nostoc commune* Vaucher ex Born. et Flah.	Paper mill wastewater	CYA149
17	*Nostoc ellipsosporum v. violacea* Rao, C.B. (after Rao, C. B.)	Paper mill wastewater	CYA150
18	*Nostoc linkia* v. (Roth) Born. et Flah. (after Frémy)	Paper mill wastewater	CYA151
19	*Nostoc spongIforme v. tenue* Rao, C. B. (after Rao, C.B.)	Tree bark	CYA152
20	*Oscillatoria formosa* Bory	Paper mill wastewater	CYA153
21	*Phormidium autumnale (Ag.) Gom. (after Gomont)*	Solid wastes deposits	CYA154

22	*Phormidium fragile (Menegh.) Gom.*	Tree bark	CYA155
23	*Scytonema tolypothrichoides* Kutz.(after Kossinskaja)	Paper mill wastewater	CYA156
24	*Tolypothryx byssoidea* (Berk.) Kirchn.	Tree bark	CYA157
25	*Tolypothryx distorta* v. *penicillata* (Ag.) Lemm.	Tree bark	CYA158
26	*Tolypothryx rechingeri* (Wille) Geitler (after Wille)	Paper mill wastewater	CYA159
27	*Westiellopsis prolifica* Janet (after Janet)	Paper mill wastewater	CYA160

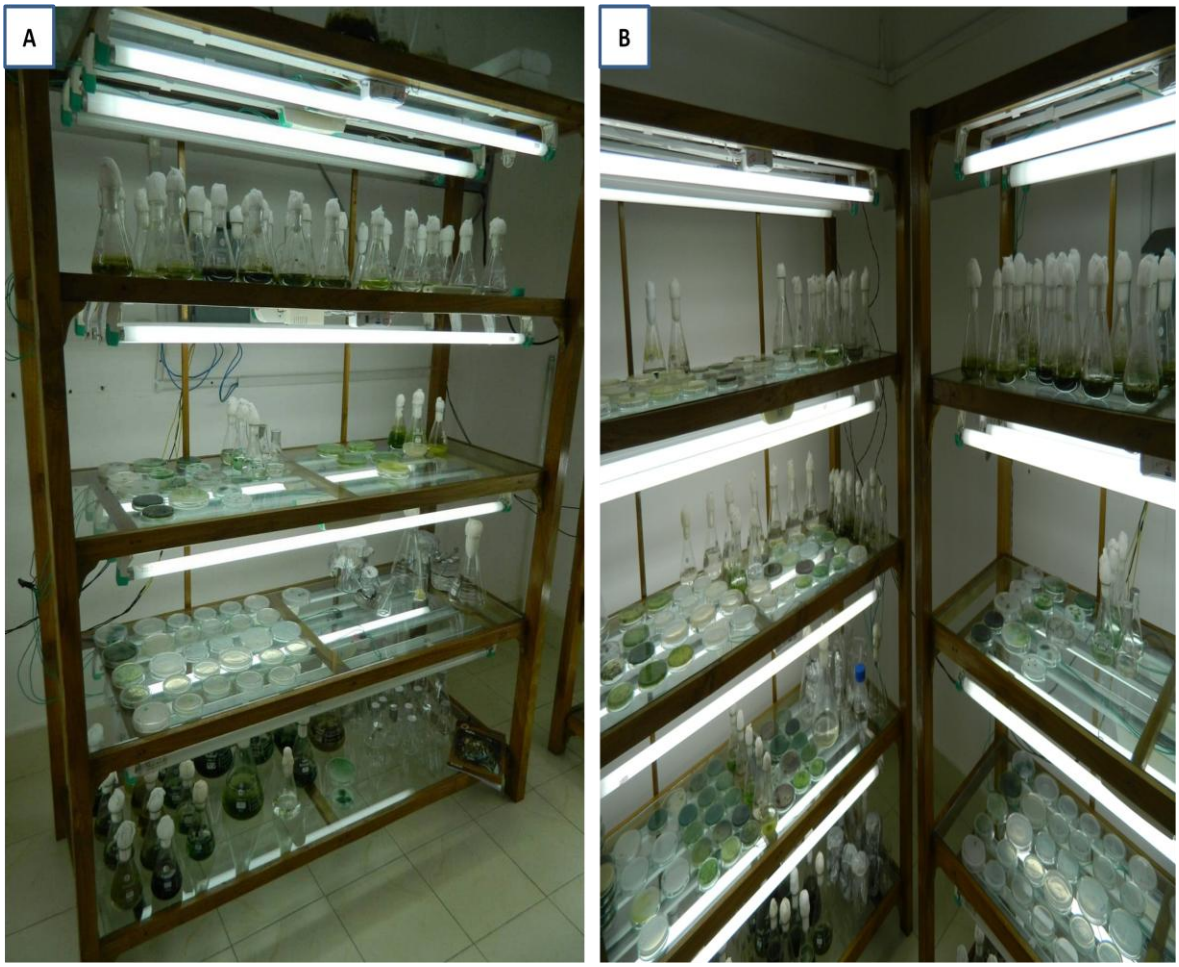

Plate 8.2 Cyanobacterial isolates submitted to the Algal Repository

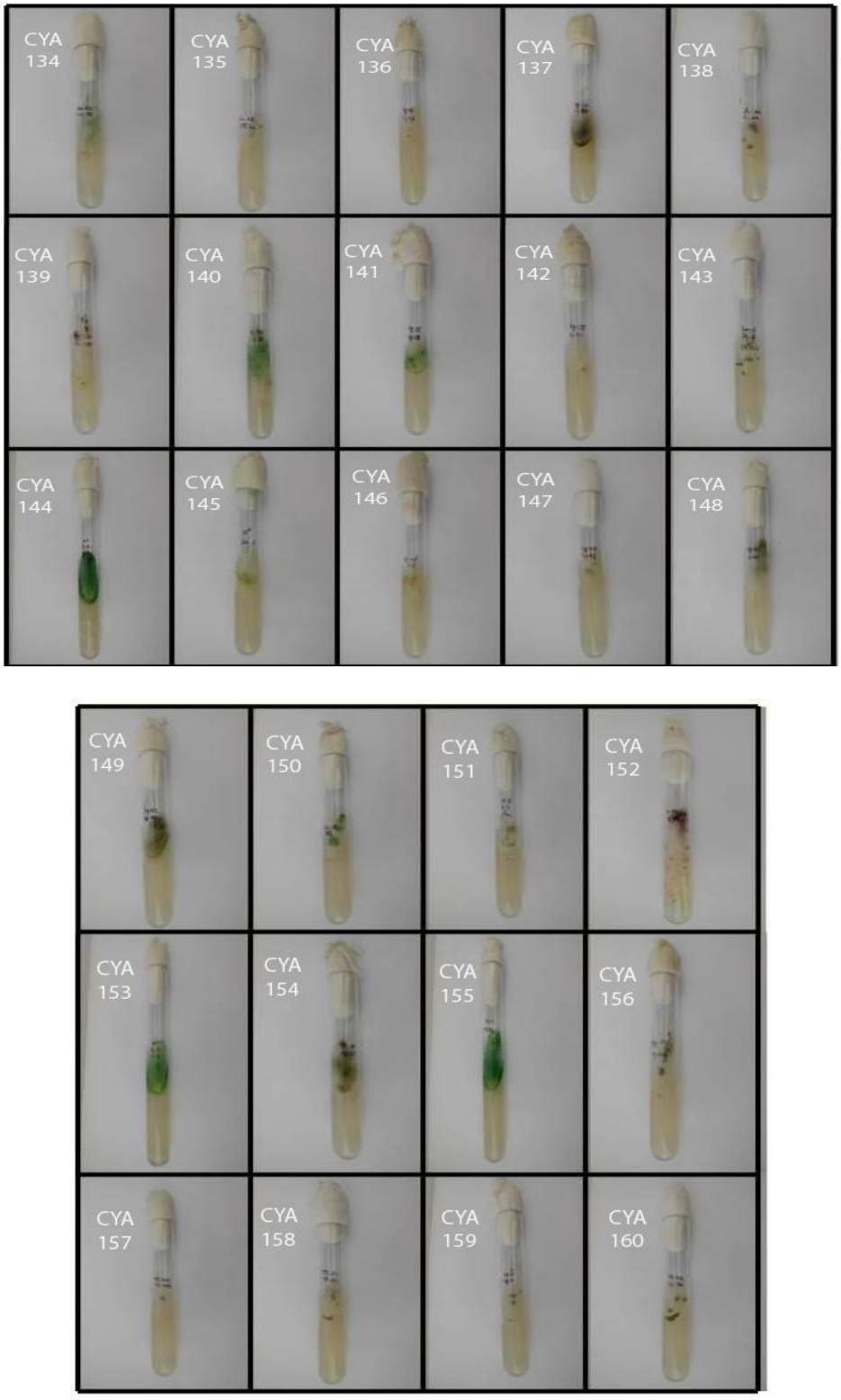

Plate 8.3 Algal repository in the Department of Ecology and Environmental Science, Assam University, Silchar.