ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Morphological and molecular characterization of potential cyanobacteria isolated from Loktak Lake

Ojit Singh Keithellakpam[1*], Indrama Thingujam[1], Gunapati Oinam[1], Avijeet Singh Oinam[1], Minerva Shamjetshabam[1], Silvia Chungkham[1], Indira Wangkhem[1], Bidyababy Thiyam[1], Sarabati Kangjam[1], Subhalaxmi Sharma Aribam[1], Thadoi Angom[1], Romi Khangembam[1], Tiwari Onkar Nath[1] and Sharma Gauri Dutt[2]

[1*,1]Freshwater Cyanobacterial and Microalgal Repository, Microbial Resources Division, Institute of Bioresources and Sustainable Development (A National Institute of DBT, Govt. of India), Takyelpat, Imphal-795001, Manipur, INDIA

[2]Department of Life Science and Bio-informatics, Hargobind Khurana School of Life Sciences, Assam University, Silchar-788011, Assam, INDIA

Manuscript Info

Manuscript History:

Received: 15 April 2015
Final Accepted: 22 May 2015
Published Online: June 2015

Key words:

Cyanobacteria, Loktak Lake, Morphology, 16S rRNA, Phylogeny, North-East India

Corresponding Author

Ojit Singh Keithellakpam

Abstract

In the present study, a total of ten (10) potential cyanobacteria isolated from Loktak Lake belonging to five (05) strains of *Nostoc* spp., three (03) strains of *Phormidium* spp., one (01) strain of *Anabaena* and one (01) strain of *Calothrix* sp. were investigated for their morphological characteristics and their phylogenetic relationship. These strains showed high phycobiliproteins content, extracellular ammonium excretion and nitrogenase activity when preliminary screening was conducted. A combination of morphological and 16S rRNA gene approach has been used for the first time to attest the phylogenetic affiliation of these potential strains. Intermingling of *Nostoc* and *Anabaena* species in 16S rRNA gene indicates that the two taxa are not clearly separated at the genetic level and may be polyphyletic in origin. In contrast to this, clustering of *Calothrix* and *Nostoc* species in the same clade attests the taxonomic coherence of these species. However, *Phormidium* showed greater phylogenetic diversity with all phylogenetic analyses. Distance matrix of 16S rRNA sequence showing similarities was ranged from 0.011-1.348. Despite a few minor incongruities, 16S rRNA gene depicted greater similarity with morphological taxonomy. Molecular characterization of cyanobacterial strains in the present study was to demonstrate their grouping and closeness of among them.

INTRODUCTION

The North-Eastern region of India has been described as a biodiversity hotspot for different kinds of flora and fauna unique. The semi-tropical climatic condition augmented with high annual rainfall has played a crucial role enhancing the biodiversity richness that supports luxuriant growth of cyanobacteria as well. Loktak Lake, rich in biodiversity and the lifeline of Manipur valley has been recognized as a wetland of International Importance (Ramsar site no. 463). Cyanobacteria have been traditionally classified on the basis of their morphological and physiological characteristics (Desikachary 1959; Geitler, 1932). During their long and slow evolution, they have achieved huge diversity both in morphology and genetics, ranging from simple unicellular organisms to complex filamentous organisms (Whitton, 1992). These characteristics make it difficult to resolve their phylogenetic relationships and elucidate taxonomic classification (Liu et al., 2003). For long time, mainly morphological

characteristics were taken into account for a taxonomical classification of cyanobacteria (Rippka et al., 1979; Schopf, 2000). Among the molecular methods, the analysis of the 16S rRNA gene sequences has proved to be a useful tool for exploring phylogenetic relationships among cyanobacteria (Han et al., 2009; Komarek, 2010; Li et al., 2008; Pan et al., 2008; Saker et al., 2009; Willame et al., 2006, Zapomelova et al., 2010). 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 conservative nature of 16S rRNA gene, its universal distribution and the vast availability of sequence information in public databases (Genbank, EMBL, DDBJ and RDP) make it the marker of choice for taxonomical studies (Weisburg et al., 1991). With the advance of molecular studies based mainly on the 16S rRNA gene sequence, the taxonomy of the group, especially of Nostocaleans, has been widely discussed. Current taxonomic revisions tend to be conducted by a multidisciplinary (polyphasic) approach including molecular, morphological, physiological, cytological, toxicological and ecological data.

This study has provided genetic information on potential cyanobacterial isolates from Loktak Lake environment for the first time, since only morphological descriptions existed previously. Preliminary screening of these strains was done based on their biochemical components such as pigment composition, extracellular ammonium excretion and nitrogenase activity. Based on the above data, the present investigation was focus on the molecular study for the grouping and closeness of strains.

Materials and Methods

Strains and growth conditions

The present studied ten (10) cyanobacterial strains were obtained from Freshwater Cyanobacterial and Microalgal Repository (National facility created by the Department of Biotechnology, Government of India with reference No. BT/PR11323/PBD/26/171/2008 dated 31-03-2009), Institute of Bioresources and Sustainable Development (IBSD), Imphal, Manipur, India which were isolated from Loktak Lake, the largest freshwater wetland in the North-Eastern region of India. Five (05) strains of *Nostoc* spp., three (03) strains of *Phormidium* spp., one (01) strain of *Anabaena* and one (01) strain of *Calothrix* sp. were investigated for their morphological characteristics and their phylogenetic relationship. The study of morphology of the strain was carried out using trinocular research microscope (NIKON Eclipse 80*i*) and Carl Zeiss fluorescence microscope, Axio Scope A1 coupled with Carl Zeiss Imaging Systems 32 software AxioVision 4.7.2 followed by taxonomical characterization referring to standard monograph (Desikachary, 1959; Komarek and Anagnostidis, 2005). Prior to genomic DNA isolation, strains were grown in sterile BG11 medium (Stanier et al., 1971). Cultures were synchronised by altering light/dark periods of 14:10 h condition maintained at 28±2ºC in light intensity of 54-67 µmol photons $m^{-2}s^{-1}$ provided by cool white fluorescent tubes.

DNA extraction and amplification

Fresh cyanobacterial biomass (exponentially growth phase) was subjected for isolation of genomic DNA according to the Xanthogenate-SDS (XS) extraction protocol (Tillett and Neilan, 2000) with slight modifications. After isolation of genomic DNA, quantification and analysis of quality was necessary to ascertain the approximate quantity of DNA obtained and the suitability of DNA sample for further analysis. Amplification of the 16S rRNA gene was carried out by PCR using primers (IDT-Integrated DNA Technologies) forward primer 536f (5'-GTGCCAGCAGCCGCGGTRATA-3') and reverse primer 1488R (5'-CGGTTACCTTGTTACGACTTCACC-3') (Nubel et al., 1997). The PCR mixture contained 5 µl of 1X reaction buffer, 5 µl of 200 µM of each dNTPs, 1.5 µl of 0.3 µM of each primer, 0.25 µl of 5U *Taq* polymerase, 2 µl (50 ng) of DNA with 34.75 µl of sterile double distilled water. Total reaction volume was 50 µl. The PCR reaction was started as initial denaturation step for 5 min at 95°C followed amplification with by 27 cycles of cyclic denaturation for 1 min at 95°C, 1 min at 55°C for annealing and 1 min at 72°C for extension. The final extension of 10 min at 72°C. Subsequently, the PCR amplicons were migrated at 80V for 1 h on 2% (w/v) agarose gel contained 1X TAE buffer (Tris-Acetate-EDTA buffer). The amplified product was visualized and documented using a Vilber Lourmat gel documentation system with Quantum-Capt software.

Sequence alignment and phylogenetic analyses

Search of the nucleotide sequences in the database GenBank homologous to the sequenced genes (16S rRNA sequences) of studied strains of cyanobacteria was performed with sequence information available in the National Centre for Biotechnology Information (NCBI) data base using nucleotide BLAST (BLASTN) program (Altschul et al., 1990). The hits with highest identities and closest homology were selected. Sequences were aligned using the ClustalW (Thompson et al., 1994) and the phylogenetic tree was constructed by the maximum parsimony (MP) (Eck and Dayhoff, 1966) and neighbor-joining (NJ) (Saitou and Nei, 1987) method with high bootstrap values (Felsenstein, 1985) and Kimura 2-parameter distance correction in the molecular evolutionary genetics analysis

software, MEGA 4.0 software (Tamura et al., 2007). Bootstrap analysis was carried out on 1000 replicates. For neighbor-joining measures, a tree using systematically taxon order dependent break ties with Kimura 2-parameter method (Kimura, 1980) was used.

To examine the phylogenetic relationship, the cyanobacterial 16S rRNA sequences obtained were aligned together with known 16S rRNA sequences of 41 other cyanobacteria of filamentous heterocystous and non-heterocystous group and also with that of *Synechococcus elongatus* PCC 6301 as an outgroup.

Results and Discussion

The strains of cyanobacteria investigated in this study and their morphological characteristics were listed in Table 1. The gel image showed band of amplified products and all the strains produced a single band of similar (Fig 1).

Report of sequence identities of cyanobacterial strains in this study based on nucleotide-nucleotide BLAST was presented in Table 2. Partial 16S rRNA gene sequences of the strains deposited in the GenBank database with accession numbers was also presented in Table 2. Pairwise similarity analysis between the partial 16S rRNA gene sequences of the cyanobacterial strains using NCBI BLASTN software revealed that *Phormidium* sp. KF953515 showed maximum identity (99%; E-value = 0) with *Phormidium* sp. (AB003169.1). The strains *Nosoc* sp. KF953516 and *Nostoc* sp. (AM711541.1) showed 99% maximum identity with E-value = 0 followed by the strains *Nosoc* sp. KF953517 and *Nostoc* sp. (HE974997.1) showing 96% maximum similarity (E-value = 0). The strains *Nosoc commune* KF953518 and *Nostoc commune* (HF678489.1) showed 99% maximum identity with E-value = 0 followed by the strains *Calothrix* sp. KF953519 and *Calothrix* sp. (AB325535.1) showing 97% maximum similarity (E-value = 0). *Phormidium* sp. KF953520 and *Phormidium* sp. (AM398775.1) showed 99% maximum similarity (E-value = 0). *Nostoc* sp. KJ511782 and *Nostoc* sp. (JX421696.1) shared 98% maximum similarity (E-value = 0) followed by the strains *Nostoc muscorum* KF953521 and *Nostoc muscorum* (AY218828.1) showed 99% maximum similarity (E-value = 0). Similarly, *Anabaena* sp. KF953522 and *Anabaena* sp. (AY422691.1) showed 98% maximum similarity (E-value = 0) followed by *Phormidium* sp. KF953523 and *Phormidium* sp. (HM217077.1) showed 87% maximum similarity (E-value = 0).

Distance matrix of 16S rRNA sequence showing similarities between strains was also generated as shown in Table 3 and was ranged from 0.011-1.348. *Nostoc commune* KF953518 showed a smaller genetic distance of 0.011 with *Nostoc muscorum* KF953521 and resulted the clustering of both in a same clade in the phylogenetic tree. Other *Nostoc* sp. KF953517 and *Nostoc* sp. KJ511782 also showed a genetic distance of 0.029 and were the sister strains clustering in same clade and evolving from a single ancestor. *Phormidium* sp. KF953515 showed genetic distance of 0.212 and 0.010 with the ancestors *Phormidium* sp. KF953523 and *Phormidium* sp. KF953520 and was evolved in separate clade.

Phylogenetic trees from both neighbor-joining and maximum parsimony analyses were similar in the placement of the sequences and their close relatives (Fig 2 & 3). In maximum parsimony strain, *Nostoc* sp. KF953517 was closely related to *Nostoc* sp. CCAP 1453/35 which topology was supported with a high bootstrap value (93%). *Calothrix* sp. KF953519 shared a common ancestor relationship with *Calothrix brevissima* IAM M-249 and *Calothrix* sp. PCC7101. *Nostoc* sp. KF953516 shared close relationship with *Nostoc* sp. PCC9231 with bootstrap value 93%. *Nostoc muscorum* KF953521 and *Nostoc* sp. PCC7423 grouped together, sharing 16S rRNA gene similarity with a high bootstrap value (100%). *Nostoc commune* BTA-67 a common ancestor relationship with *Nostoc* sp. PCC7423 and *Nostoc spongiaeforme* Ind42 with a bootstrap value of 93%. *Anabaena* sp. KF953522 and *Anabaena azotica* FACHB-118 grouped together, shared 100% 16S rRNA gene similarity. Interestingly, *Phormidium* sp. KF953515 was outside the branches that contained other *Phormidium* spp. strains in the trees constructed using NJ method while this strain was grouped with *Phormidium pristley* ANT.L52.6 strain in the MP tree (bootstrap value of 100%). Similarly, *Nostoc* sp. BTA-80 shared a common ancestor with the *Nostoc spongiaeforme* Ind42 and *Nostoc commune* KF953518 (in NJ tree) and with the cluster that contained strains belongs to several heterocystous genera in MP tree. *Phormidium* sp. KF953523 shared most common ancestors with two separate but strongly supported monophyletic groups which consist of other *Phormidium* spp. *Phormidium* sp. KF953523 was not closely related to any sequences from the NCBI GenBank database and branched separately from other known cyanobacteria (in MP and NJ tree).

Taxonomic revisions are required to be done by a multidisciplinary approach including molecular, morphological, physiological, cytological and ecological (Hoffmann and Gugger, 2003; Komarek and Kastovsky, 2003; Suda et al., 2002). The classification of cyanobacteria has routinely relied on morphological characteristics which are not always trustworthy, as they may show variation depending on culturing and environmental conditions (Nayak et al., 2007) and lead to misidentifications (Komarek and Anagnostidis, 1989). These problems of traditional morphological classification, together with the lack of molecular data, pose serious hindrances for taxonomy and systematics of cyanobacteria (Hayes et al., 2007; Komarek, 2010).

The maximum identity score of the 16S rRNA sequence of the present study was found in the ranged from 87-99% and E-value as 0. The clusters are well supported by bootstrap analysis and partly reflect the morphological similarity of the organisms. The preliminary results indicate that some strains of *Nostoc* were genetically closer whereas other strains of *Nostoc* were genetically separate and closer with *Anabaena* and *Phormidium*. Only one strain of *Phormidium* sp. (KF953523) got grouped separately. The clustering of morphologically distinct groups in the present study was supported by previous work (Giovannoni et al., 1988) describing the phylogeny of cyanobacteria obtained from 16S rRNA gene sequences. The present attempt was to identify whether morphological characters of these strains on which the taxonomic identity is based was genetically strong and stable. All heterocyst forming cyanobacteria studied appeared to share a common ancestor. Morphologically *Anabaena* and *Nostoc* strains formed an extremely tight cluster which was highly supported by bootstraps values of MP and NJ trees.

In distance matrix of 16S rRNA sequence, the distances in the trees created by 16S rRNA sequencing revealed that the evolutionary relationships of the cyanobacterial strains were not clear. Turner (1997) and Wilmotte (1994) have also reported the uncertain grouping of the strains belong to these genera by 16S rRNA gene sequence analysis. The system of molecular identification of cyanobacteria when used together with traditional morphological characterization should continue to assist in the accurate delineation of novel strains and the presentation of an amended system for cyanobacterial classification. 16S rRNA gene remains for its availability the most used and useful tool in molecular phylogeny at the genus level. Sequences of the 16S rRNA gene serve especially for delimitation of genera inside groups of cyanobacteria (Sihvonen et al., 2007; Wilmotte and Herdman, 2001). The incorporation of molecular techniques has been of great help to delimit and characterize genera and higher-level taxa, allowing to understand the diversification processes within cyanobacteria, which in turn has affected their classification. At the species level, however, the use of the 16S rRNA gene is not generally sufficient by itself for species delimitation because the similarity values are often high (above 98%) and phylogenetic analyses allow already the recognition of significant differences. The 16S rRNA gene analysis (phylogeny and percentage of similarity) is not enough for species recognition and delimitation, thus stressing the necessity of a polyphasic approach with morphological, molecular and ecological data.

Literature on cyanobacteria of the Loktak Lake are very few. The study on diversity of cyanobacteria from this lake were contributed earlier by Chingkheihunba and Arvind (2011), Singh et al. (2012), Tiwari and Singh (2005). Studies on cyanobacteria from this freshwater lake is expected to provide a wealth of information that is not only academically rewarding but also very useful in planning mass cultivation techniques oriented towards the biotechnological exploitation of these organisms since they were found to be high in biopigment content and could be used as biofertilizers. Their utilization for the production of high value-added products would be of great economic importance in the future.

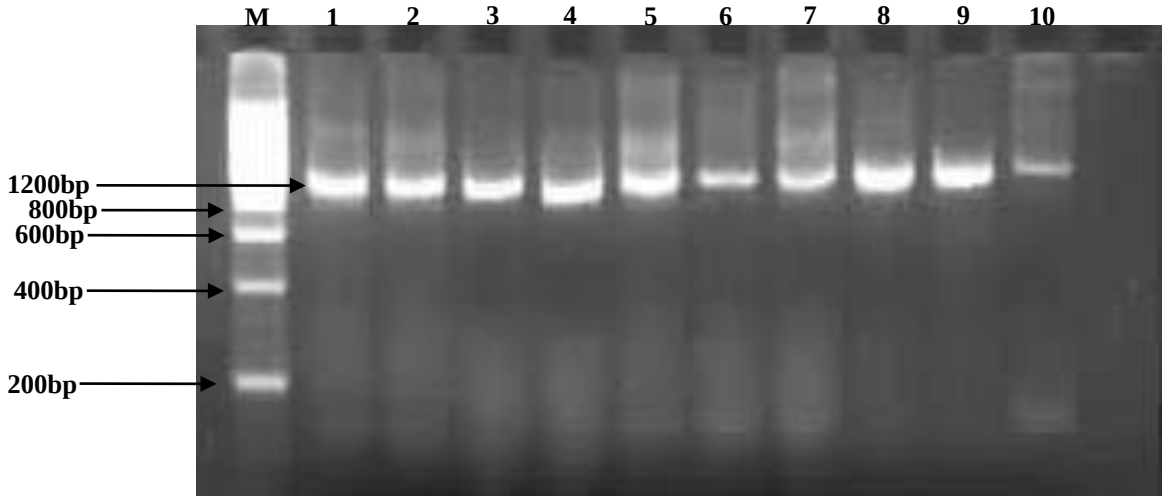

Fig 1. Gel image of amplified products of 16S rRNA PCR

M-200bp DNA ladder; **1**-*Phormidium* sp. BTA-52; **2**-*Nostoc* sp. BTA-60; **3**-*Nostoc* sp. BTA-61; **4**-*Nostoc commune* BTA-67; **5**-*Calothrix* sp. BTA-73; **6**-*Phormidium* sp. BTA-75; **7**-*Nostoc* sp. BTA-80; **8**-*Nostoc muscorum* BTA-950; **9**-*Anabaena* sp. BTA-964; **10**-*Phormidium* sp. BTA-1048

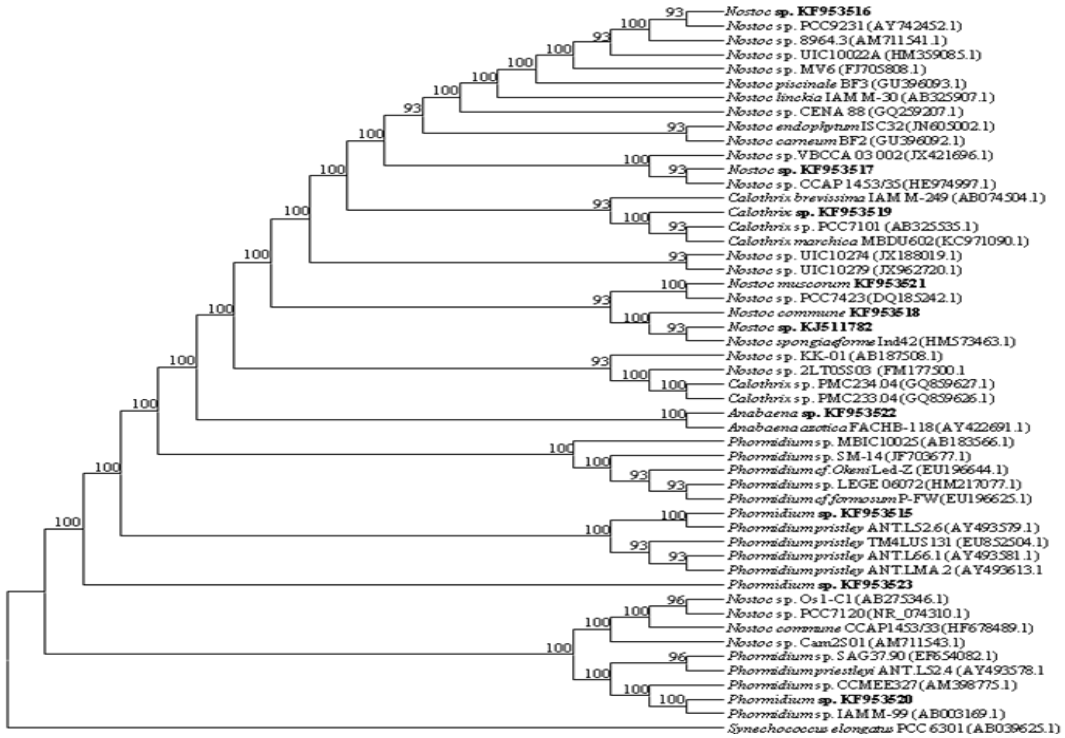

Fig 2. Maximum parsimony tree based on partial 16S rRNA gene sequences. The tree includes 10 sequences of cyanobacterial strains determined in the present study (bold) and 41 sequences from NCBI Genbank database. The Genbank accession number of each taxa is in parentheses. Bootstrap values (1000 replicates) are placed at the nodes of the branches if they were equal to or greater than 50%

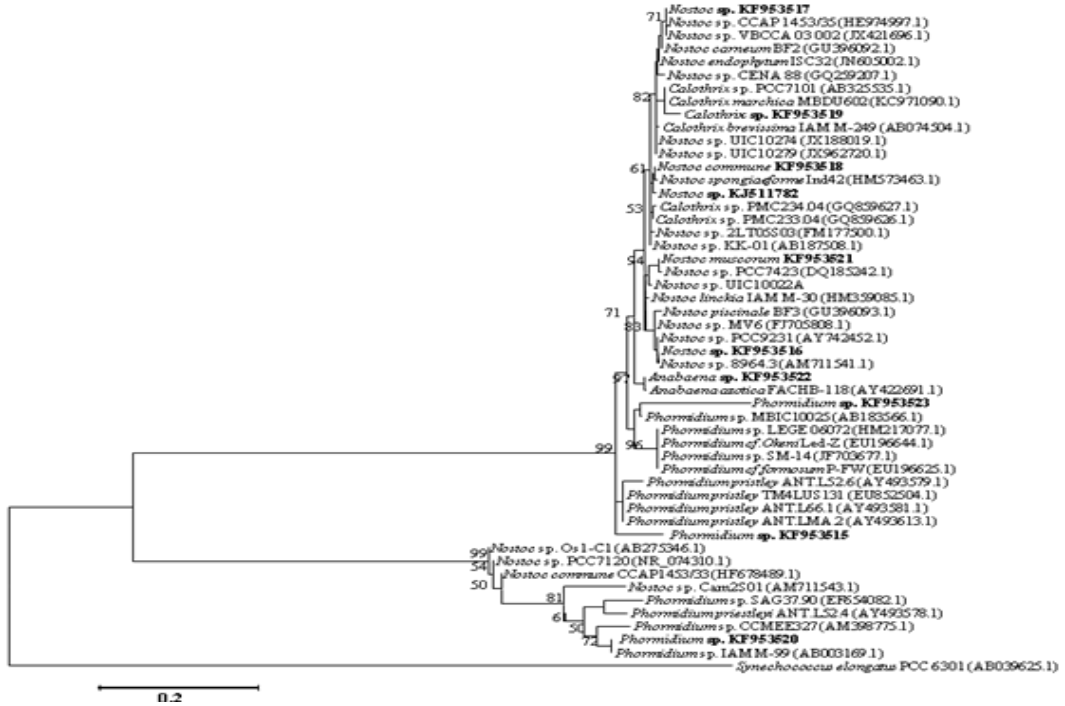

Fig 3. Neighbor-joining (kimura) tree based on partial 16S rRNA gene sequences. The tree includes 10 sequences of cyanobacterial strains determined in the present study (bold) and 41 sequences from NCBI Genbank database. The Genbank accession number of each taxa is in parentheses. Bootstrap values (1000 replicates) equal to or greater than 50% are indicated at the nodes. The scale bar indicates 0.2 substitutions per nucleotide position

Table 1. Morphological characteristics of cyanobacterial strains from Loktak Lake, Manipur, India and yield and quality of isolated DNA

Cyanobacterial strains	**Morphological features**	**DNA concentration (ng μl^{-1})**	**A_{260}/A_{280}**
Phormidium sp. BTA-52	Dark green, initially bottom attached and later submerged, membranous biomass, bent, indistinct sheath, cell longer than broad	312.8	1.86
Nostoc sp. BTA-60	Light green, initially bottom attached and later reticulate biomass formation, flexuous**,** barrel or spherical cells with both intercalary and terminal heterocysts which is sub-spherical	429.9	1.81
Nostoc sp. BTA-61	Dark brown, submerged biomass, reticulate and floccose biomass, flexuous, cell quadratic with spherical heterocyst	423.9	1.83
Nostoc commune BTA-67	Dark green, bottom attached, floccose biomass, filament densely entangled, barrel shape cell with spherical shaped heterocyst	116.9	1.79
Calothrix sp. BTA-73	Dark brown, initially bottom attached and later floating, floccose biomass, slightly bent, barrel shaped cell, basal and spherical heterocyst	310.3	1.84
Phormidium sp. BTA-75	Dark green, floating, floccose biomass, straight and granulated at the joints, long apical cell with a calyptra hyaline and colourless sheath	413.5	1.78
Nostoc sp. BTA-80	Light brown, initially bottom attached and later floating, floccose biomass, flexuous, intercalary and terminal spherical heterocyst	294.2	1.8
Nostoc muscorum BTA-950	Dark green, initially bottom attached and later submerged, glomerate biomass, flexuous, barrel shaped cell, intercalary and terminal heterocyst	367.9	1.75
Anabaena sp. BTA-964	Dark green, submerged, floccose biomass, flexuous, hyaline and colourless sheath, barrel shaped cell, spherical heterocyst	385.3	1.87
Phormidium sp. BTA-1048	Dark green, floating and attached on side, reticulate biomass, slightly bent, distinct sheath**,** quadrate cell	401.4	1.74

Table 2. Sequence identities of cyanobacterial strains in this study based on nucleotide-nucleotide BLAST (blastn) of NCBI

Cyanobacterial strains	Query coverage %	NCBI Accession no.	Closest BLAST match (accession no.)
Phormidium sp. BTA-52	100	KF953515	*Phormidium* sp. (AB003169.1)
Nostoc sp. BTA-60	100	KF953516	*Nostoc* sp. (AM711541.1)
Nostoc sp. BTA-61	100	KF953517	*Nostoc* sp. (HE974997.1)
Nostoc commune BTA-67	99	KF953518	*Nostoc commune* (HF678489.1)
Calothrix sp. BTA-73	100	KF953519	*Calothrix* sp. (AB325535.1)
Phormidium sp. BTA-75	99	KF953520	*Phormidium* sp. (AM398775.1)
Nostoc sp. BTA-80	100	KJ511782	*Nostoc* sp. (JX421696.1)
Nostoc muscorum BTA-950	99	KF953521	*Nostoc muscorum* (AY218828.1)
Anabaena sp. BTA-964	100	KF953522	*Anabaena* sp. (AY422691.1)
Phormidium sp. BTA-1048	100	KF953523	*Phormidium* sp. (HM217077.1)

Table 3. Distance matrix for 16S rRNA sequences showing similarities between cyanobacterial strains

Strains	KF953515	KF953516	KF953517	KF953518	KF953519	KF953520	KJ511782	KF953521	KF953522	KF953523
Phormidium sp. (KF953515)										
Nostoc sp. (KF953516)	0.091									
Nostoc sp. (KF953517)	0.108	0.055								
Nostoc commune (KF953518)	0.093	0.034	0.042							
Calothrix sp. (KF953519)	0.093	0.042	0.055	0.032						
Phormidium sp. (KF953520)	0.010	0.025	0.015	0.028	0.019					
Nostoc sp. (KJ511782)	0.101	0.047	0.029	0.030	0.047	1.212				
Nostoc muscorum (KF953521)	0.095	0.034	0.051	0.011	0.036	1.239	0.040			
Anabaena sp. (KF953522)	0.128	0.060	0.062	0.036	0.066	1.239	0.055	0.036		
Phormidium sp. (KF953523)	0.212	0.189	0.189	0.187	0.200	1.348	0.175	0.191	0.187	

Acknowledgements
The research leading to these results has received funding from the Indian Council of Agricultural Research (ICAR), Government of India. The authors thank Director, IBSD-DBT, Imphal, Manipur, India for providing laboratory facilities and required infrastructure.

Conflict of interest
The authors declare that they have no conflict of interest.

References
Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990): Basic local alignment search tool. J. Mol. Biol. 215(3): 403-410.
Chingkheihunba, A. and Arvind, K.S. (2011): Molecular typing and distribution of filamentous heterocystous cyanobacteria isolated from two distinctly located regions in North-Eastern India. World J. Microbiol. Biotechnol. 27: 2187-2194.
Desikachary, T.V. (1959): Cyanophyta, Monographs on Algae, Indian Council of Agricultural Research, New Delhi, India, pp. 656.
Eck, R.V. and Dayhoff, M.O. (1966): Atlas of protein sequence and structure, National Biomedical Research Foundation, Silver Spring, Maryland.
Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4): 783-791.
Geitler, L. (1932): Cyanophyceae. In: Kolkwitz R. (eds) Rabenhorst s Kryptogamen flora von Deutschland, Osterreich und der Schweiz XIV, Akademische, Verlagsgesellschaft, Leipzig, pp. 1-1196.
Giovannoni, S.J., Turner, S., Olsen, G.J., Barns, S., Lane, D.J. and Pace, N.R. (1988): Evolutionary relationships among cyanobacteria and green chloroplasts. J. Bacteriol. 170(8): 3584-3592.
Han, D., Fan, Y. and Hu, Z. (2009): An evaluation of four phylogenetic markers in *Nostoc*: implications for cyanobacterial phylogenetic studies at the intrageneric level. Curr. Microbiol. 58: 170-176.
Hayes, P.K., El Semary, N.A. and Sanchez-Baracaldo, P. (2007): The taxonomy of cyanobacteria: molecular insights into a difficult problem. In: Brodie J., Lewis J. (eds) Unravelling the algae: The past, present and future of algal systematic, CRC Press/Taylor & Francis Group, Boca Raton, pp. 93-101.
Hoffmann, L. and Gugger, M. (2003): Morphological and molecular characterisation of a stigonematalean cyanobacterium isolated from Spanish cave. Algol. Stud.109: 259-65.
Kimura, M. (1980): A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol.16 (2):111-120.
Komarek, J. (2010): Modern taxonomic revision of planktic nostocacean cyanobacteria: a short review of genera. Hydrobiologia 639(1): 231-243.
Komarek, J. and Anagnostidis, K. (1989): Modern approach to the classification system of Cyanophytes 4-Nostocales. Algol. Stud. 56: 247-345.
Komarek, J. and Anagnostidis, K. (2005): Cyanoprocaryota 2.Teil, Oscillatoriales. In: Budel K., Krienitz L., Gartner G., Schagerl M. (eds) Suesswasserflora von Mitteleuropa 19/2, Elsevier, Spektrum Akademische Verl., Heidelberg, pp. 758.
Li, R., Wilhelm, S.W., Carmichael, W.W. and Watanabe, M.M. (2008): Polyphasic characterization of water bloom forming *Raphidiopsis* sp. (Cyanobacteria) from central China. Harm. Algae 7(2): 146-153.
Liu, X.J., Chen, F. and Jaing, Y. (2003): Differentiation of *Nostoc flagelliforme* and its neighboring species using fatty-acid profiling as a chemataxonomic tool. Curr. Microbiol. 47(6): 467-474.
Nayak, S., Prasanna, R., Prasanna, B.M. and Sahoo, D.B. (2007): Analysing diversity among Indian isolates of *Anabaena* (Nostocales, Cyanophyta) using morphological, physiological and biochemical characters. World J. Microbiol. Biotechnol. 23: 1575-1584.
Nubel, U., Garcia-Pichel, F. and Muyzer, G. (1997): PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl. Environ. Microbiol. 63(8): 3327-3332.
Pan, X.J., Chang, F.Y., Kang, L.J., Li, G.B., Li, D.H., Liu, Y.D., Shen, Y.W. and Wei, Z.H. (2008): Morphological characteristics and phylogenetic relationship of *Anabaena* species from Lakes Dianchi and Erhai, China. Hydrobiologia 614(1): 353-362.
Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y. (1979): Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111: 1-61.
Saitou, N. and Nei, M. (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4(4): 406-425.

Saker, M., Moreira, C., Martins, J., Neilan, B. and Vasconcelos, V.M. (2009): DNA profiling of complex bacterial populations: toxic cyanobacterial blooms. Appl. Microbiol. Biotechnol. 85: 237-252.

Schopf, J.W. (2000): The fossil record: tracing the roots of the cyanobacterial lineage. In: Whitton B.A., Potts M. (eds) The Ecology of cyanobacteria, Kluwer Academic Publishers, Netherlands, pp. 13-35.

Sihvonen, L.M., Lyra, C., David, P., Fewer, D.P., Rajaniemi-Wacklin, P., Lehtimaki, J.M., Wahlsten, M. and Sivonen, K. (2007): Strains of the cyanobacterial genera *Calothrix* and *Rivularia* isolated from the Baltic sea display cryptic diversity and are distantly related to *Gloeotrichia* and *Tolypothrix*. FEMS Microbiol. Ecol. 61: 74-84.

Singh, K.O., Oinam, G. and Tiwari, O.N. (2012): New record of potential cyanobacteria from Indian region falling Indo-Burma biodiversity hotspots (North-East region of India) and partial characterization for value additions. Philipp. J. Sci. 141(1): 57-66.

Stanier, R.Y., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. (1971): Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35(2):171-205.

Suda, S., Watanabe, M.M., Otuska, S., Mahakant, A., Youngmanitachi, W., Noparatnaraporn, N., Liu, Y. and Day, J.G. (2002): Taxonomic revision of water-bloom-forming species of Oscillatorioid cyanobacteria. Int. J. Syst. Evol. Microbiol. 52: 1577-95.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007): MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24(8): 1596-1599.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids Res. 22(22): 4673-4680.

Tillett, D. and Neilan, B.A. (2000): Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. J. Phycol. 36(1): 251-258.

Tiwari, O.N. and Singh, H.T. (2005): Biodiversity of cyanobacteria in Loktak Lake and rice fields of Manipur, India having acidic properties. Proc. Natl. Acad. Sci. India. 75(B) III: 209-213.

Turner, S. (1997): Molecular systematics of oxygenic photosynthetic bacteria. Plant Syst. Evol. 11: 13-52.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. and Lane, D.J. (1991): 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173: 697-703.

Whitton, B.A. (1992): Diversity, ecology and taxonomy of the cyanobacteria. In: Mann N.H., Carr N.G. (eds) Photosynthetic prokaryotes, Plenum press, New York, pp. 1-51.

Willame, R., Boutte, C., Grubisic, S., Wilmotte, A., Komarek, J. and Hoffmann, L. (2006): Morphological and molecular characterization of planktonic cyanobacteria from Belgium and Luxembourg. J. Phycol. 42: 1312-1332.

Wilmotte, A. (1994): Molecular evolution and taxonomy of the cyanobacteria. In: Bryant D.A. (eds) The molecular biology of cyanobacteria, Kluwer Academic Publishers, Netherlands, pp. 1-25.

Wilmotte, A. and Herdman, M. (2001): Phylogenetic relationships among the cyanobacteria based on 16S rRNA sequences. In: Boone D.R., Castenholz R.W. (eds) Bergey's manual of systematic bacteriology, Springer, New York, pp. 487-493.

Zapomelova, E., Rehakova, K., Jezberova, J. and Komarkova, J. (2010): Polyphasic characterization of eight planktonic *Anabaena* strains (cyanobacteria) with reference to the variability of *Anabaena* populations observed in the field. Hydrobiologia 639(1): 99-113.