Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Taxonomic evaluation of Cyanobacteria using Morphological Characters and Molecular phylogeny using cpcB and cpcA Gene Sequencing

Yogesh Pawar, Kruti Dave, Samruddha Phadnis and Ganesh Iyer
Department of Life Science, Ramnarain Ruia College, Matunga, Mumbai-400019
Email: yogeshpawar44@gmail.com

Abstract

A biodiversity study was conducted in Mumbai, Thane and Raigad districts. Six cyanobacterial genera (*Cyanobacterium, Phormidium, Plantothrix, Spirulina, Synechococcus* and *Synechocystis*) were selected for taxonomic study using morphological characters and molecular taxonomic identification. It was observed that morphological characters need a supporting sequencing data for taxonomic study. Phycocyanin gene was selected so that any contamination other than cyanobacteria can be eliminated. A phylogenetic tree was constructed using MEGA 6 software and observed that the genera *Phormidium, Planktothrix* and *Spirulina* are polyphyletic and need an reassessment in the class.

Keywords: Cyanobacteria, Morphology, Phycocyanin gene sequencing, Phylogeny, Taxonomy,

Introduction

Cyanobacteria, also called as blue green algae, are the most primitive photosynthetic organisms on the earth. Due to its arrangement of genetic material they were often classified in bacterial group but they are now classified under Kingdom plantae because of the presence of photosystem I and II (Thajuddin, N and Subramanian, G., 2005). They are found in various habitats such as lakes, rivers, ponds, rain water puddles, moist soil, and on tree barks. They are not only free living but are also found in association with plants and other green algae.

Cyanobacteria are also used as biofertilizers in agriculture (Mishra, U. and Pubbi, S, 2004) due to their ability to fix atmospheric nitrogen which gets available to crop plants. They also have a good amount of pigment Phycocyanin, blue in colour, bearing many medicinal uses. Phycocyanin is also used as a natural food colouring agent in food industries (Pandey, V.D., Pandey, A. and Sharma, V., 2013). Due to their high value products Cyanobacterial research has raised in past dacades. Taxonomy of cyanobacteria becomes a problem in identifying different strains ideal for specific purpose. For this reason morphological characters are being supported with molecular biology data for confirmation in identification.

Phycocyanin is the primary pigment of cyanobacteria which is also found in Red alga as secondary pigment. This pigment is absent in any other organism and hence has been selected for sequencing and phylogenetic analysis. Phycocyanin is used as molecular marker for taxonomic purpose by most of the researchers throughout the world (Premanandh J et al, 2006) (Reehana, N., 2013). During this work we have identified the selected genera and species based on their morphological characters and then sequenced to get confirmation on it. The sequencing data supports the morphological identification.

Materials and Methods

Sample collection, inoculation and growth

Cyanobacterial samples were collected from various places in Thane, Nasik and Raigad districts. *Phormidium uncinatum* was collected from Shelu river, district Thane, *Planktothrix perornata* was collected from Igatpuri, district Nasik and Kelavli, district Raigad in a small puddle in monsoon season, *Spirulina subsalsa* was found growing in moist place near Bhatsa reservoir in Khardi, district Thane, *Synechocystis sp* LSCB01 and *Synechococcus elongatus* were collected from the terrace on Ruia College, district Mumbai in a plant pot with damp soil, *Cyanobacterium stanieri* was collected from Khnandeshwar, district Raigad, Maharashtra in a polluted water body beside the Highway. Monoculture preparation was achieved by inoculating these cyanobacterial samples on BG11/Zarrouck's agar plates and liquid medium. *Spirulina subsalsa* and *Cyanobacterium stanieri* were grown on Zarrouk's agar (2%) and liquid medium pH 9.0 described by Gami B, Naik, A. and Patel B (2011) with some modifications (**Table 1**). All other genera were grown on BG11 (HiMedia M1541-500G) Agar Medium (2%) and liquid medium (pH 7.5). All the chemicals used were of analytical grade and purchased from HiMedia. Cultures were illuminated with 3000 lux true lights with the photoperiod of 12:12 hrs light – dark cycle and at a temperature of 27-30 ^{o}C. Each of the sample were photographed using Labomed Lx300 microscope and calibration were done using software Pixel Pro.

Table 1.: Media composition to prepare Zarrouk's Medium.

	Constituent	Amount to be added per litre
1	Sodium bicarbonate ($Na_2HPO_{4)}$	18 gms
2	Sodium nitrate ($NaNO_{3)}$	2.5 gms
3	Potassium phosphate dibasic (K_2HPO_4)	0.5 gms
4	Potassium sulphate (K_2SO_4)	1.0 gm
5	Sodium chloride (NaCl)	1.0 gm
6	Calcium chloride ($CaCl_2$)	0.04 gm
7	Ehtylene di-amine tetra-acetic acid disodium salt (Na_2-EDTA)	0.08 gm
8	Magnesium sulphate ($MgSO_4$)	0.2 gm
9	Ferrous sulphate ($FeSO_4$)	0.01 gm
10	A_5 Metal Solution*	1 mL

A_5 Metal solution*

	Constituent	Amount to be added per litre
1	Boric acid (H_3BO_3)	2.86 gms
2	Ammonium molybdate [$(NH_4)_6Mo$]	0.02 gm
3	Manganese chloride ($MnCl_2$)	1.8 gms
4	Copper sulphate ($CuSO_{4)}$	0.08 gm
5	Zinc sulphate ($ZnSO_4$)	0.22 gm

Isolation of Genomic DNA

Genomic DNA was isolated using protocol mentioned by Prabha T. R. et.al. (2013). The sample was obtained either from a liquid growth culture or from solid agar medium. Sample was homogenized in 1 ml of extraction buffer (200 mM Tris HCl pH 7.5, 25 mM EDTA, 250mM NaCl and 0.5% SDS) and collected in 1.5 ml eppendorf tube and left at room temperature for 30 mins. All the tubes were centrifuged at 13000 rpm for 1 min and the supernatant was collected in fresh eppendorf. To this equal volume of cold Phenol:Chloroform (1:1) was added and vortexed briefly to mix the content. This was again centrifuged at 13000 rpm for 2 mins at 4 ^{o}C. The supernatant was collected in fresh eppendorf tube and 300 µL of Chilled chloroform was added. The content was mixed thoroughly by inverting the tube repeatedly. The mixture was centrifuged again at 13000 rpm for 2 mins at 4 ^{o}C. This step was repeated again and the supernatant was transferred in a fresh eppendorf tube. Now 300 µl of cold iso-propanol was added and the content was mixed gently by inverting the tube. All the tubes were kept at -20 ^{o}C for 30 mins and centrifuged at 13000 rpm for 5 mins. The supernatant was discarded and the pellet was washed with 100 µl cold 70% ethanol. Again centrifuged at 13000 rpm for 5 mins and the pellet was dried and dissolved in 30 µ of TE buffer pH 8.0. About 1-5 µl of this DNA was used in PCR amplification using PCβF and PCαR primers specific for phycocyanin gene.

PCR Amplification using c-PC primers

PCR was performed using 2X PCR Taq mix ordered from HiMedia (Cat. No. MBT061-R) and primers PCβF (GGCTGCTTGTTTACGCGACA) and PCαR (CCAGTACCACCAGCAACTAA) were obtained from GeneOmbiotech, Pune. Molecular biology grade water was purchased from HiMedia (ML024-500ML). A reaction mixture of 50 µl was achieved using **table 2**. The reaction mixture was placed in BioEra Neo Peltier Thermocycler 16 well model. PCR programme was set as per **Table 3** for PCR amplification of phycocyanin gene.

Table 2.: Preparation of 50 µl reaction mixture for PCR

No.	Component	Volume
1	2X PCR Taq Mix	25 µl
2	PCβF Forward Primer	4 µl
3	PCαR Reverse Primer	4 µl
4	Template DNA	1-5 µl (approx 250 ng)
5	Molecular Biology grade water	To make 50 µl

Table 3.: PCR Programme for Phycocyanin gene amplification

Segment	Temperature	Hold Time	Up Speed	Down Speed	Total Cycles
1	95 °C	10 Mins			
2 Three step Cycle	94 °C	1 min	1 °C/ S	1 °C/ S	35 Cycles
	50 °C	1min 10 Sec	1 °C/ S	1 °C/ S	
	72 °C	1 min	1 °C/ S	1 °C/ S	
3	72 °C	10 mins			
4	4 °C	Until further Use			

The PCR product was run onto 1 % Agarose gel (HiMedia) and stained with Ethidium Bromide (HiMedia). 100 bp DNA ladder purchased from HiMedia was loaded to identify the PCR product size. The gel was visualized and photographed in a self made Gel documentation system. The sample was then analyzed spectrophotometrically using absorbance at 260nm/280nm ratio to check the purity and quantity of the PCR product. Further purification of PCR product and sequencing was carried out at GeneOmbiotechnology Ltd., Baner, Pune.

Purification and Sequencing of PCR product

The products were purified by using a geneO-spin PCR product Purification kit (GeneOmbio technologies, Pune; India) and were directly sequenced using an ABI PRISM BigDye Terminator V3.1 kit (Applied Biosystems, USA). The sequences were analyzed using Sequencing Analysis 5.2 software. BLAST analysis was performed at BlastN site at NCBI server.

Results

Morphological and molecular taxonomic comparison

Morphological identification:

***Phormidium uncinatum* (Ag) Gomont** **PLATE I- 2**

Synonym: *Phormidium autumnale* Gomont

Reference:

Desikachary, T.V., 1959, Cyanophyta, ICAR, Pg. 276, Pl 43, Fig. 1, 2, Pl. 45, fig. 9, 10.

Description:

Thallus expanded, dark green, adhered to the soil, thin firm, filaments straight slightly curved at the apex, sheath mucilaginous, trichome blue-green, very slightly constricted at the cross walls, cells 4-5 µ broad, ends briefly attenuated, slightly capitates, cells 2-4 µ long, cross walls frequently granulated.

***Planktothrix perornata* Skuja** **PLATE I-3**

Reference:

Desikachary, T. V., 1959, Cyanophyta, ICAR, Pg. 205, Pl. 41, Fig. 7

Description:

Trichomes blue-green, dark due to large granules, erect stiff, apices briefly attenuated and bent, well constricted at the cross walls, trichomes narrower, 9-10 µm broad, cells 2.4-3.4 µm long with small gas vacuoles.

The species was found growing along with *Arthrospira platensis* in the Titwala river, Dist. Thane, Maharashtra.

*This species resembles with *Planktothrix (Oscillatoria) perornata* f. attenuata Skuja as per the description provided by Desikachary, T.V.

***Spirulina subsalsa* Oerst. Ex Gomont** **PLATE I- 6,7**

Reference:

Desikachary, T.V., 1959, Cyanophyta, ICAR, Pg. 193, Pl. 36 fig. 3, 9

Description:

Trichome 1.6 µ broad, blue-green to somewhat violet, mostly irregularly densely spirally coiled, spirals sometimes loosely coiled, forming a bright blue-green thallus, spirals adhering to each other, 3.1 µ broad.

The alga was found floating as a thin layer in a puddle in Khandeshwar along with Cyanobacterium stanieri.

***Synechocystis* sp. Strain LSCB 01** (PCC6803) **PLATE I- 4**

Synonym: *Synechocystis pevalekii* Ercegovic

Reference:

Desikachary, T. V., 1959, Cyanophyta, ICAR, Pg. 145, Pl. 25 Fig. 11

Description:

Thallus indefinite, among other algae, cells spherical, after division hemispherical, 2.2-2.5 µ broad, single or two together, contents blue-green homogeneous.

The alga was found growing in a puddle with *Phormidium uncinatum* in Shelu.

***Cyanobacterium stanieri* Rippka et Cohen-Bazire 1983** **PLATE I- 5**

Reference:

Rippka, R. & Cohen-Bazire, G. (1983). The Cyanobacteriales: a legitimate order based on the type strain Cyanobacterium stanieri. Annales de l'Institut Pasteur, Microbiologie 134B: 21-36.

Description:

Cells solitary or in groups of two after division, sometimes in irregular clusters but never in colonies, more or less cylindrical, rod shaped with rounded ends, straight or slightly arcuate, cell content homogeneous, cells 2.25-3.42 µ broad & 3.87-5.47 µ long, no envelop sheath, content in the centre of the cell clear, hyaline, towards periphery bright blue-green, rapidly dividing cell division by binary fission.

***Synechococcus elongatus* Nag.** **PLATE I- 1**

Reference:

Desikachary, T.V., 1959, Cyanophyta, ICAR, Pg. 143, Pl. 25, fig. 7,8

Description:

Cell cylindrical, elongated, 1.2-1.7 µ broad, 3-4 µ long, single or two cells together, contents homogenous, light blue-green.

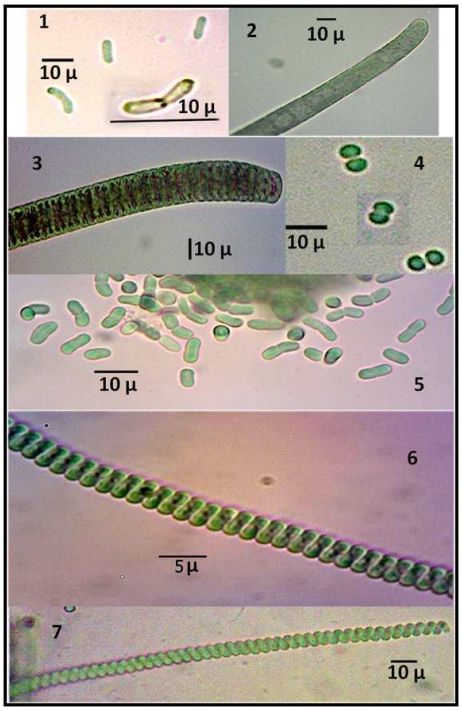

Plate I: **1.** *Synechococcus elongatus* strain LSCB 05 , 2. *Phormidium uncinatum* Shelu 01, 3. *Planktothrix perornata* IGTP 11, 4. *Synechocystis sp*. LSCB 01, 5. *Cyanobacterium stanieri* strain KD 02, 6,7. *Spirulina subsalsa* KD 01

Molecular taxonomy

The sequencing results of all the cyanobacterial genera were analyzed using BLAST search in NCBI website. Sequences of these seven genera are mentioned in **table 4**. The four cyanobacteria *Spirulina subsalsa*, *Planktothrix perornata* and *Synechococcus elongatus* were identified morphologically and confirmed by sequenceing data. The other three cyanobacteria *Phormidium uncinatum*, *Synechocystis* sp. PCC 6803 and *Cyanobacterium stanieri* were morphologically identified as *Oscillatoria proteus*, *Synechocystis* sp. and *Cyanobacterium aponimum*. Using the sequencing data these species were named accordingly with the strain name as per the geographical distribution. The sequences were then uploaded in NCBI via Bankit. The accession numbers for each of the above sequences were received from genbank (**Table 5**).

Table 4: Phycocyanin gene sequences of six cyanobacterial genera

***Phormidium uncinatum* Shelu 01**

CTACGCTATCCTGGCTGGTGACTCCAGCGTATTGGATGACCGCTGCCTGAATGGTCTGCGCGAAACCTATCAAGCTCTGGGTACTCCCG
GTTCTTCCGTAGCTGTTGGCGTTTCCAAGATGAAAGAAGCCGCCATCGCAATTGCTAATGACCCCAGTGGCATCACCCAAGGCGATTGC
AGCTCTTTGATCTCTGAAGTAGCTAGCTACTTCGATCGCGCTGCTGCTGCTGTTGCTTAATTTAGATTGAGTGGTGAGTGAGGATCGTCT
CCTGCTCCCAAAGCTGCTCTAAATGCGATATTTCGCCCATTAGTTAGCAAATTTTGCAAAGAATTCCGGAGATATCTAAAACATGAAGA
CCCCTATTACCGAAGCAATTGCCGCAGCAGATACTCAAGGTCGTTTTCTGAGCAACAGCGAATTGCAAGCAGTTCGCGGTCGTTTGGA
ACGCGCAGCCGCTAGCATGGAAGCTGCTCGCGCTCTGACCAACAACGCTCAAAAGCTAGTTGATGGTGCAGCTAACGCGGTATATCAA
AAGTACCCTTACACCACCCAACAACAAGGTTCTAACTT

***Planktothrix perornata* IGTP 11**

GCTTGAATGGCTTGCGCGAAACCTACCAAGCTCTAGGTACTCCCGGCGGTTCCGTAGCAGCAGGCGTGCAAAAAATGAAGGAAGCCGC
GATCGCGATCGTTAGCGATACCAACGGCATCACTCGTGGTGATTGCAGCTCCTTGATCTCTGAAATCGGTGGCTACTTCGACCGCGCAG
CATCGGCAGTAGGCTAAAAAAGCCTTGCGGGTTATATCTGTAAAGACGCGACGAGTCGCATCTGGACAAAACAAACTTGTTACGAATA
CCAAAGCAAAACACTAGGGAGATACCTCAACAATGAAAACCCCTTTGACTGAAGCAGTCACAGCCGCCGATTCTCAAGGCCGCTTCCT
GAGCAGCACCGAACTTCAAGTTGCTTTCGGTCGCTTCCGCCAAGCCACCGCTGGTTTAGAAGCAGCCAAATCTTTGACAGCTAACGCTC
AGCGTTTGACCGATGGTGCAGCCCAAGCAGTGTACAACAAATTCCCGTACACCACCCAAATGCAAGGCAACAACTACGCCTCCACCCA
AACTGGTAAGGCAAAGTGCGCCCGTGACATCGGCTA

***Spirulina subsalsa* KD 01**

CCAAGCTCTGGGTGTTCCCGGTGCTTCCGTCGCTGCTGGCGTGCAAAAAATGAAAGATGCTGCCATTTCTTTGGCTAACGACCCCAACG
GGATCACCCCTGGCGATTGCAGCCAACTGATCTCCGAAGTGGCCAGCTACTTTGATCGCGCTGCTGCTGCTGTGGCTTAAGCCCCTGTT
TCTTGCTCGCTTTATCAATTCAGATCTTTAGATCTAAATGTTCGGGGAATTTCCCCAACAGCACGAGACAACAATTATCCAGAATTTAG
GAGAAGCCTAGACCGATGAAAACCCCTATCACCGAAGCGATCGCAACCGCTGATTCCCAAGGCCGTTTCTTGGGCAACACCGAACTCC
AAGCTGCTAACGGTCGCTTTGAGCGTGCCCAAGCCAGCATGGAAGCTGCTCGCGCTCTGACCCAAAAGTCCCGCGACCTGATCAACGG
TGCAGCTAATGCTGTGTACCAACGGTTCCCCT

***Synechocystis* sp. LSCB 01**

GACATGGAAATCATCCTCCGCTATGTTACCTACGCAACCTTCACCGGCGACGCTTCCGTTCTAGAAGATCGTTGCTTGAACGGTCTCCG
TGAAACCTACGTTGCCCTGGGTGTTCCCGGTGCTTCCGTAGCTGCTGGCGTTCAAAAAATGAAAGAAGCTGCCCTGGACATCGTTAACG
ATCCCAATGGCATCACCCGTGGTGATTGCAGTGCTATCGTTGCTGAAATCGCTGGTTACTTCGACCGCGCCGCTGCTGCCGTAGCCTAG
TCTGGTTATTTTAAAAACCAACTTTACTCAGGTTCCATACCCGAGAAAATCCAGCTTAAAGCTGACATATCTAGGAAAATTTTCACATT
CTAACGGGAGATACCAGAACAATGAAAACCCCTTTAACTGAAGCCGTTTCCACCGCTGACTCTCAAGGTCGCTTTCTGAGCAGCACCG
AATTGCAAATTGCTTTCGGTCGTCTACGTCAAGCTAATGCTGGTTTGCAAGCCGCTAAAGCTCTGACCGACAATGCCCAGAGCTTGGTA
AATGGTGCTGCCCAAGCCGTTTATAACAAATTCCCCTACACCACCCAAACCCAAGGCAACAACTTTGCTGCGGATCAACGGGGTAAAG
ACAAGTGTGCCCGGGACATCGGCTACTACCTCCGCATCGTTACCTACTGCTTAGTTGCTG

***Cyanobacterium stanieri* KD 02**

CCTGGATCTTCTGTTGCTGTTGGCGTACAAAAAATGAAAGATGCTGCTTTAGCAATTGCTAACGACACCAACAACATTACCCTTGGTGA
TTGTAGCCAATTAATGGCTGAAGTTGCTACCTATTTTGATCGTGCTGCTGCTGCAGTAGCGTAATTAATAGCTACCACACAAGTTAAAA
AATCATTTCGTTAACAAAAACACAAATTTTAAGGAGAATATTAGACAATGAAAACCCCTTTAACTGAAGCAGTATCCGCAGCAGATTC
TCAAGGTCGTTTCCTCAGCAGCACCGAAATCCAAACCGCTTTTGGTCGTTTCCGTCAAGCTAGTGCTAGCTTAACCGCCGCTAAATCTTT
AACCGAAAACGCTCAACGCTTAGTTGATGGTGCAACAAACGCTGTATACAACAAATTCCCTTACACCACCTCCACCCCCGGTGCTAACT
ATGCTTCTACCGCTGAAGGTAAAGCAAAATGTTCTCGTGACATCGGTTACTACC

***Synechococcus elongatus* LSCB 05**

ACGCTACCTTGGCTGGCGACTCCAGTGTGTTGGATGACCGTTGCTTGAATGGTCTGCGCGAAACCTACCAAGCTTTGGGAACTCCTGGT
TCTTCCGTGGCTGTTGGCGTTCAAAAAATGAAAGAAGCCGCCATTGCCGTTGCCAACGACCCCAATGGGATCACCCCTGGCGATTGCA
GCTCTTTAATGTCTGAGTTGGCTGGTTACTTTGACCGGGCCGCTGCTGCCGTAGCCTAAATCCCCCTGGATGACTACACTTAAATATCTT
TCTTGACCGCTTTCTAAGACACGAAACTTATTAAGGGAGAAATCTCAAGACTATGAAGACCCCGATTACTGAAGCGATTGCCGCCGCT
GATACCCAAGGTCGTTTCCTCAGCAACACCGAACTCCAGGCTGCTGATGGTCGGTTTAAGCGGGCTGCTGCTAGCTTGGAAGCCGCTCG
TGCTTTGACCAGCAATGCCCAAAGCTTGATCGATGGTGCTGCTTCTGCTGTGTATCAGAAGTTTCCTTACACCACCTCCATGCAAGGTTC
TCAATACGCTTCAACTCCCGAAGGCAAAGCCAAATGTGCCCGGGATATCGGTTACTACCTGCGGATGGTGACCTACTGCTTAGTTG

cpcB gene cpcA gene

Table 5. Accession numbers of sequences uploaded in NCBI

No.	Organism	Accession Number
1	*Phormidium uncinatum* Shelu 01	KJ094043
2	*Planktothrix perornata* IGTP 11	KJ094042
3	*Spirulina subsalsa* KD01	KF994611
4	*Synechocystis sp*. LSCB 01	KJ018112
5	*Cyanobacterium stanieri* strain KD02	KM249029
6	*Synechococcus elongatus* strain LSCB05	KM249030

Phylogenetic analysis

Phylogenetic tree was constructed using free software MEGA 6.0. The sequences were aligned with other related strains to observe the phylogenetic relationship. Total of 33 nucleotide sequences of Phycocyanin gene were aligned in MEGA 6.0

using Clustal W and Phylogenetic analysis was found out. The phylogenetic tree was constructed by using Neighbour joining method (Saitou N. and Nei M., 1987), the bootstrap method was used to test the phylogeny with the 500 replications of the Bootstrap (Felsenstein J., 1985). The evolutionary distances were computed using p-distance method and all the gaps and missing data were eliminated completely (**Fig. II**).

Fig: III Evolutionary relationships of Cyanobacterial strains
The evolutionary history was inferred using the Neighbor-Joining method (Saitou N. and Nei M., 1987). The optimal tree with the sum of branch length = 1.95203576 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein J., 1985). 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 (Nei M. and Kumar S., 2000) and are in the units of the number of base differences per site. The analysis involved 33 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 195 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S., 2013).

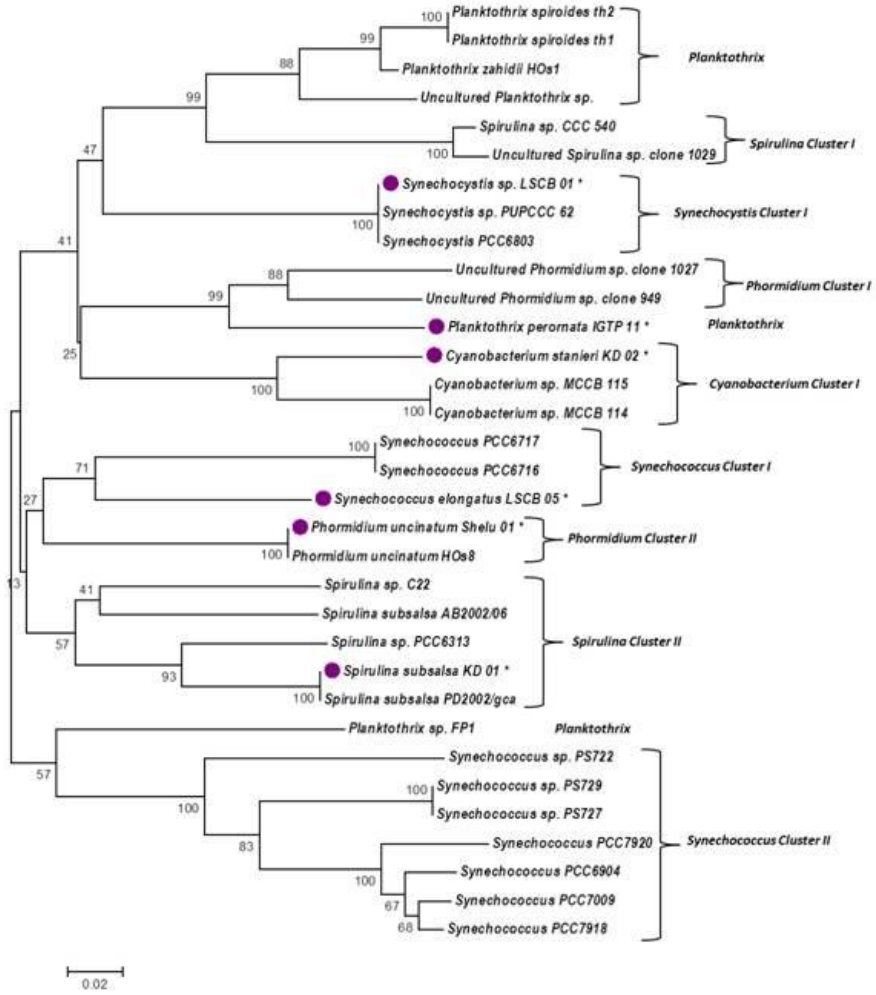

Fig: II Evolutionary relationships of Cyanobacterial strains

Conclusion

The genomic DNA extraction protocol described by Prabha T. R. et.al is a rapid and superlative method for DNA extraction from Cyanobacteria. From the above study it can be concluded that the phycocyanin gene is a reliable marker for identification and taxonomy purpose. From the phylogenetic analysis of the cyanobacterial strains with respect to Phycocyanin gene, it can be clearly observed that the genera *Cyanobacterium* and *Synechocystis* are monophyletic. Other three genera i.e. *Synechococcus, Spirulina* and *Phormidium* form polyphyletic clusters. *Planktothrix perornata* IGTP 01 is the first entry in NCBI database and hence no reference sequence was found for the species. The genus forms a poyphyletic cluster but no homology is observed. *Planktothrix perornata* IGTP 01 forms a cluster with uncultured *Phormidium* clone and hence more data is required for this particular species to form a cluster with the genus *Planktothrix*, otherwise the species should be included in other genera with which it may form a cluster. Due to polyphyletic cluster formation the family and

orders are also not monophyletic, hence it is strongly recommended to reassess the class Cyanobacteria with special reference to Phycocyanin cpcB and cpcA gene and intergenic spacer sequence region.

Discussion

The DNA extraction using the protocol described by Prabha, T. R., et al. is a useful and rapid protocol. This protocol is used for extraction of DNA in fungi and therefore it has great use in breaking down the strong fungal exoskeleton to extract the nuclear DNA. The same protocol when used for cyanobacterial cells, it was observed that DNA was extracted rapidly. The yield of DNA was also quite well as compared to other protocols and kits available in the market. Therefore it is recommended to use this protocol to reduce the cost of chemicals and time required for the DNA extraction purpose. In this study Phycocyanin gene was sequenced for phylogenetic analysis and its benefit lies in the symbiotic associationship between Cyanobacteria and Bacteria. It is quite possible that during the 16srDNA sequencing maximum sequence of bacteria is sequenced than the cyanobacteria. Most of the scientists recommend the gene to be sequenced and a database to be prepared for cyanobacterial phylogeny and taxonomy (Reehana, N. et al., 2013; Neilan, B. A. et al., 1995; Premanandh, J. et al., 2006). A comparison between 16s rDNA and cpcB and A genes was carried out by Robertson, B. R. et al. and observed somewhat similar results and therefore phycocyanin gene is a reliable marker gene for phylogeny (Robertson, B. R. et al., 2001)

Acknowledgements

We acknowledge the sincere help rendered by Mr. Yashwant Chavan, GeneOmbiotechnoloy Pvt Ltd., in amplifying and sequencing the Phycocyanin gene.

References

Desikachary, T.V., 1959, Cyanophyta, ICAR.

Felsenstein J., 1985, Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39:783-791.

Gami B, Naik, A. and Patel B, 2011, Cultivation of Spirulina species in different liquid media, J. Algal Biomass Utln., 2 (3): 15– 26

Mishra, U. and Pubbi, S., 2004, Cyanobacteria. A Potential Biofertilizer for Rice, Resonance, Genral Article, Pg. 6-10.

Nei M. and Kumar S., 2000, Molecular Evolution and Phylogenetics, Oxford University Press, New York.

Neilan, B. A., Jacobs, D and Goodman, A. E., 1995, Genetic Diversity and Phylogeny of Toxic Cyanobacteria Determined by DNA Polymorphisms within the Phycocyanin Locus, *Applied and Environmental Microbiology*, 61(11): 3875–3883

Pandey, V.D., Pandey, A. and Sharma, V., 2013, Biotechnological application of Cyanobacterial Pycobiliproteins, Int. J. Curr Micobiol. App. Sci, 2(9): 89-97.

Prabha, T.R, Revathi K, Vinod M.S, Shanthakumar S.P, Bernard P, 2013, A simple method for total genomic DNA extraction from water moulds., Current Science, 104(3):345-347

Premanandh J, Priya B, Teneva I, Dzhambazov B, Prabaharan D, Uma L., 2006, Molecular characterization of marine Cyanobacteria from the Indian subcontinent deduced from sequence analysis of the phycocyanin operon (cpcb-IGS-cpcA) and 16S-23S ITS region, J Microbiol., 44(6):607-16.

Reehana,N., Parveez Ahamed A., Mubarak Ali,D, Suresh,A., Arvind Kumar,R., Thajuddin, N. 2013, Structure Based Computational Analysis and Molecular Phylogeny of C- Phycocyanin Gene from the Selected Cyanobacteria, International Science Index, International Journal of Biological, Life Science and Engineering, 7(1):12-16

Rippka, R. & Cohen-Bazire, G., 1983, The Cyanobacteriales: a legitimate order based on the type strain Cyanobacterium stanieri?. Annales de l'Institut Pasteur, Microbiologie 134B: 21-36

Robertson, B. R., Tezuka, N. and Watanabe, M. M., 2001, Phylogenetic analyses of *Synechococcus* strains (cyanobacteria) using sequences of 16S rDNA and part of the phycocyanin operon reveal multiple evolutionary lines and reflect phycobilin content, *International Journal of Systematic and Evolutionary Microbiology*, 51: 861–871

Saitou N. and Nei M., 1987, The neighbor-joining method: A new method for reconstructing phylogenetic trees, Molecular Biology and Evolution, 4:406-425.

Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S., 2013, MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30: 2725-2729.

Thajuddin, N. and Subramanian, G., 1992, Survey of cyanobacterial flora of the southern east coast of India. Bot. Mar., 35, 305–311.