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Suitability of Phycocyanin gene Sequences for the Identification of Cyanobacterial taxa belonging to Oscillatoriales

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ABSTRACT: Cyanobacteria, known as blue green algae have evolved over billions of years to survive in a variety of environments, including those with extreme temperatures, pH, and salinities. Identifying cyanobacteria can be challenging due to their morphological diversity, and the presence of similar-phenotypic with variations of genotypic characters. Most common problems encountered when identifying cyanobacteria are (1) Morphological variation of same taxa may differ in the natural environment and in laboratory conditions; (2) Taxonomic revisions are made frequently as cyanobacterial taxonomy is constantly revolving; (3) Cryptic species like those are morphologically similar but genetically distinct, (4) Cyanobacterial growth are influenced by the environmental conditions like light, temperature, pH leading to phenotypic variations and Lack of comprehensive reference databases. In this present study, modern genetic, cyto-morphological, and ecological methodologies must be included and integrated for cyanobacterial taxonomy in order for their contemporary systematics to be developed. Phycocyanin (PC) gene sequences were used taxonomic resolution between 7 different Oscillatorial species and their Phylogenetic analysis; primary-secondary structure prediction, Ramachandran plot and homology modeling were studied. The diversity was revealed after processing the phycocyanin gene sequence and was deposited in GenBank (NCBI). From this study, interference in strain level variations among different species belonging to same genus of Oscillatoriales were elaborated using PC gene sequences which can be considered as a suitable molecular marker for identification of cyanobacteria.

Keywords: Molecular Taxonomy, Phycocyanin, Phylogenetic resolution, Structure prediction, Oscillatoriales.

INTRODUCTION

Cyanobacteria is an ancient class of prokaryotic photosynthetic organisms with a worldwide distribution existing in soils, thermal springs, freshwater and marine ecosystems, as well as the polar regions (Garcia-Pichel, 2009; Whitton, 2012; Abed *et al.,* 2009). These organisms generate a variety of physiologically active chemicals with carcinogenic, immunosuppressive, antifungal, antibacterial, anticancer, and antitumor activities as secondary metabolites (Whitton and Potts 2012; Gupta *et al*, 2020). This established their significant potential as sources of chemicals for biotech, pharmaceutical, and food industries. Despite their vast application, cyanobacterial nomenclature has undergone multiple significant step modifications since early 2000s as a result of revolution in genome sequencing, initiating their designation as prokaryotes continuing through a plenitude of new taxonomic descriptions and re-classifications (Alvarenga *et al.,* 2017). Indeed morphology appear lacking in today's contemporary identification, their systematic grouping method is completely reliant on phenotypic (and scarcely ecological) features. Its evolutionary patterns, variable cyto-morphological properties, and quick adaptation have evolved in different ways in all groups, complicating the taxonomic classification of cyanobacteria. The stability and taxonomic significance of many criteria found in cyanobacterial genomes can vary among various higher taxa. As a result, the taxonomic significance of the same traits in other clades can vary. Due to their molecular data, that doesn't overlap, even same species from different strains has varied evolutionary sequence (Muralitharan and Thajuddin 2011; Anand *et al.,* 2019). The most predominant 16S rRNA gene was used to determine phylogenetic relationships and identify cyanobacteria (Shakena *et al*., 2022; Katie Shiels *et al*., 2019). All prokaryotes have these molecules, and since their sequence varies from strain to strain, it can be used to infer evolutionary relationships. Moreover, the 16S rRNA gene sequences were found to be relatively simple to align, and a sizable collection of sequences (over 11 lakh cyanobacterial sequences as of today) has amassed, enabling strain analyses (Ludwig and Klenk 2005). Several markers, including the 16S rRNA, *nif* gene, ITS region, *rpo* gene, phycocyanin locus, and

phosphoenolpyruvate carboxylase gene, among others, have been used to study the biodiversity of cyanobacteria (Smith *et al*., 2008). Rapid improvements in genomic and alternative molecular research technologies and trends in information technologies have blended to provide an incredible quantity of information associated with molecular biology with the aid of using the utility of bioinformatics.

Cyanobacteria are the best microorganisms providing considerable quantities of Phycocyanin (PC) and its derivative Allophycocyanin. The investigation of PC gene sequence heterogeneity will be appropriate for the categorization of freshwater cyanobacteria because of the PC distributions in aquatic blue-green algae. The potential to improve taxonomic resolution from genus-level to genotype-level by utilizing molecular techniques instead of microscopic methods is one of their key benefits, which isn't always feasible to achieve *via* different strategies (Ouellette and Wilhelm 2003). C-Phycocyanin gene was first stated (Pilot & Fox, 1984), in which the oligonucleotide synthesis of alpha-beta subunits of *cpc* gene of a freshwater cyanobacterium *Agmenellum quadruplicatum* was described and also stated in *Spirulina platensis*. The *cpc* gene sequences of some *Spirulina* strains were used an important a site to analyse their phylogenetic relationships. There isn't any exact detail about the regulatory sequence and cpc operon (Yu, 2002). In this study, interference of genetic relationship between 7 freshwater cyanobacterial isolates was evaluated using phycocyanin gene sequences. The taxonomic resolution was demonstrated through its phylogenetic tree, primary and secondary structure prediction, and homology modelling of the protein sequence. These methods allowed cyanobacterial genera to be well distinguished with their genotypic and phenotypic differences. Those Phycocyanin proteins were docked with antibiotics using molecular docking, in order to determine the effect of inhibition of phycocyanin against 10 different classes of antibiotics Thus, this study determines the taxonomic interference between among different Oscillatorial strains using Phycocyanin gene, which can be developed as a molecular marker for identification of cyanobacteria.

MATERIALS AND METHODS

Culture collection. 7 Strains of Oscillatorial strain were obtained from National Repository of Marine and Cyanobacteria – Freshwater (NRMC-F), established by Department of Biotechnology (DBT), Bharathidasan University, Trichy. Antibiotics like streptomycin, ampicillin and gentamycin were selected to remove the cyanobacteria associated bacteria.

Microscopic Analysis. Light microscopy (Micros, Austria) was used to capture microphotographs of filamentous cyanobacteria, which were then identified with standard monographs of Desikachary (1959); Geitler (1932). We measured the morphology of the filament cell, including its length and width, attenuation, constriction, and terminal cell.

Extraction of DNA. Smoker and Barnum (1988) method were modified to extract DNA from cyanobacteria in which 1ml of cells are pelleted using centrifugation by decanting the medium. To the cell pellet, 500µl of STE buffer [20µl Lysozyme (20mg/ml), 20µl Proteinase K (20mg/ml) and 20µl SDS (10%) was added. They were grinded in mortar and pestle, later incubating at 70ºC for 1 hour. Equal amount of Phenol: chloroform: Isoamyl alcohol was added to the mixture and gently mixed which are then centrifuged at 7,000rpm for 7 mins to separate phases. Upper phase will be collected in a new sterile tube to which 400µl of 4M ammonium acetate and 600µl isopropanol are added. Final centrifugation at 8000 rpm for 10 minutes was given after 70% ethanol wash, then disintegrated in TE buffer and stored at -20ºC.

C-Phycocyanin gene amplification by Polymerase Chain Reaction. For PCR amplification, a universal 16S rRNA gene with forward PCβF-5'CTGCTTGTTTACGCGACA3' and reverse PCαR -5'CCAGTACCACCAGCAACTAA3' sequences were used (Neilan, 2002). The PCR reaction mixture for amplification was 30µl volume containing 15 µl of Master mix (1X), 1 µl of forward and reverse primer, 1 µl of genomic DNA and 12 µl od deionized distilled water. PCR cycle has 94ºC for 10 mins, 94 ºC for 1mins, 47 ºC for 1mins, 72 ºC for 1 min (30 cycles) and 72 ºC for 5 minutes. After completion of cycle, 5 PCR cycle includes 94ºC for 10 mins, 94 ºC for 1mins, 47 ºC for 1mins, 72 ºC for 1 min (30 cycles) and 72 ºC for 5 minutes of PCR product was loaded in 1.2% agarose gel with 10 µl ethidium bromide. The gel was recorded in a gel documentation of Bio Rad, USA (Gupta *et al*., 2020).

Sequencing and Phylogenetic Analysis of C-Phycocyanin. The sequencing was carried out for amplified samples in Eurofins Genomics, Bangalore. Blast analysis was against the Gen Bank database and Phylogenetic tree was generated using Neighbour joining algorithm (NJ) (Saitou and Nei 1987) of Mega 11 software.

Primary structure prediction. The primary structure was inferred by online program ProtParam, a tool that permits the computation of many physical and chemical properties, from the nucleotide sequence through the amino acid sequence(http://www.expasy.org/tools/protparam.html).

Secondary structure prediction. The sequences are translated to protein sequence using mega software for secondary structure prediction. The query sequence was uploaded in the alignment box and submitted to GOR IV secondary structure prediction for analysis of structure where structure prediction and compared with models are also performed.

Ramachandran plot & Homology modeling. SWISS-MODEL, a homology model building tool that models the 3D structure of C-Phycocyanin gene (Arnold *et al.*, 2006). The overall stereo chemical quality of modelled 3D structure was evaluated and validated, accuracy of the protein model through Ramachandran plot with an online software RAMPAGE. (Ramachandran *et al.*, 1963).

RESULTS AND DISCUSSION

7 strains of Oscillatoria were collected, deposited in National Repository of Microalgae and Cyanobacteria-

Freshwater (NRMCF), identification was determined and performed as per standard monographs of Desikachary (1959); Geitler (1932). Those strains were morphologically studied (Fig. 1) and also found the genus-species name for all the 7 strains using manual method (Shakena *et al.,* 2022). Smoker and Barnum's method [1988] was used to extract the genomic DNA from each strain, and Phycocyanin forward and reverse primers were used for amplification. The amplified products were confirmed along with 1 Kb molecular ladder using gel electrophoresis with 1.2% agarose, which can be visualized further on gel documentation (Fig. 2). Finally, sequences were sent for sequences and obtained sequences are deposited in NCBI database (Table 1). The morphological studies were already studied in detail on Shakena *et al.* (2022) focusing their length, breadth, presence and absence of cross wall constriction, attenuation, trichome end etc.

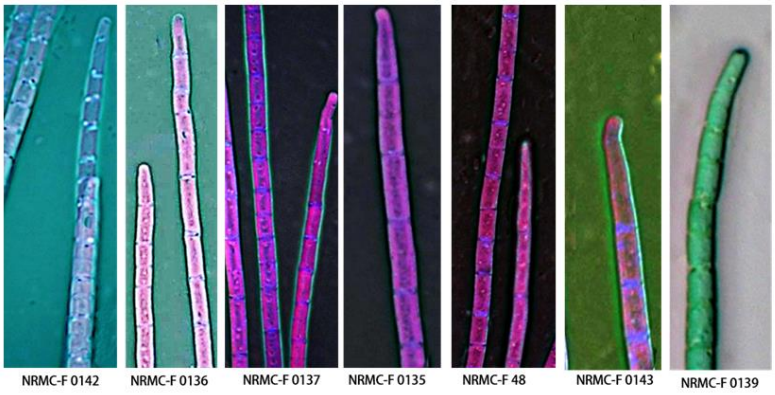


Fig. 1. Bright field microphotographs of filamentous Oscillatorial strains tested in this study.

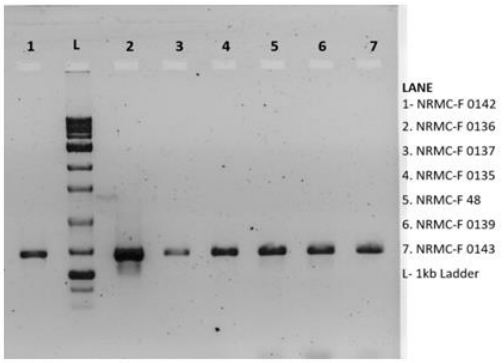


Fig. 2. PCR amplification of Cyanobacterial isolates using Phycocyanin gene primer.

Table 1: GenBank accession number for 7 different filamentous stains of *Oscillatoria.*

Strain No.	Accession Number	Strain Name
NRMC-F 0142	OM984738	*Oscillatoria calcuttensis*
NRMC-F 0136	OM984739	*Oscillatoria earlei*
NRMC-F 0137	OM984740	*Oscillatoria earlei*
NRMC-F 0135	OM984741	*Oscillatoria amoena*
NRMC-F 48	OM984742	*Oscillatoria acuta*
NRMC-F 0143	OM984743	*Oscillatoria jasorvensis*
NRMC-F 0139	ON759207.1	*Oscillatoria laetevirens*

The phycocyanin alpha and beta gene sequences of the investigated cyanobacterial species were aligned using CLUSTAL-W over similar sequences from the NCBI GenBank database. The phylogenetic tree was built using the Neighbor-Joining method (Saitou and Nei 1987). The phylogenetic tree reveals eleven strains, all of which are trichomatous types. Gene sequences of the isolates used in this experiment had a high bootstrap value, despite the reality that a bootstrap evaluation at some nodes showed a low confidence level. *Oscillatoria jasorvensis* and *Oscillatoria acuta* showed bootstrap value of 100% whereas *Oscillatoria laetevirens*, *Oscillatoria amoena* and *Oscillatoria earlei* have 99% of bootstrap value (Fig. 3). Genetic linkages may conflict with morphological classification, according to phylogenetic tree of cyanobacteria (Lyra *et al*., 2001; Iteman *et al*., 2002; Muralitharan and Thajuddin 2008). The paucity of cultures for some cyanobacterial morphospecies and insufficient morphological information from sequenced strains make it difficult to compare morphological and genetic data. Additionally, during prolonged laboratory cultivation, some strains may lose crucial characteristics like gas vesicles or colony morphology, which makes identification more difficult.

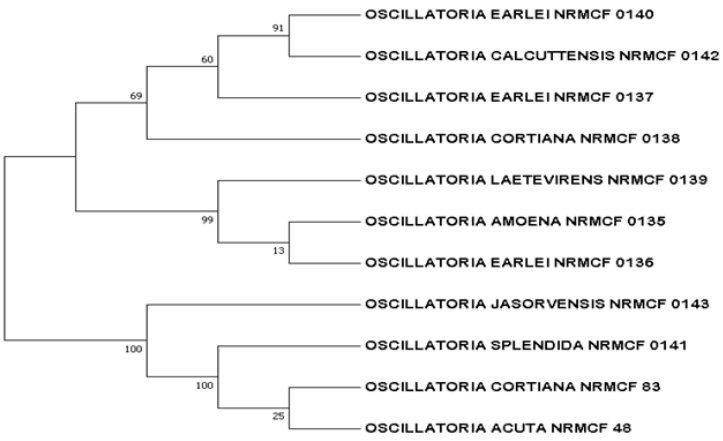


Fig. 3. Phylogenetic tree analysis of Phycocyanin gene sequences of different strains of *Oscillatoria.*

Physio-chemical factors such as the molecular weight, isoelectric point (pI), extinction coefficient, half-life, aliphatic index, amino acid property, instability index, and Grand Average of Hydropathicity (GRAVY) have been determined using the Prot Param tool of the Expasy proteomics server to analyse the primary structure prediction for the samples (Table 2).*Oscillatoria jasorvensis* NRMC-F 0143 had the most amino acid residues (354), and it had an average molecular weight of 39470.18 g mol^{-1}, according to the study's findings. *Oscillatoria cortiana* NRMC-F 0138 had 248 amino acid residues with an average molecular weight of 31521.22 g mol^{-1}. The calculated isoelectric point (PI) was higher than 7, indicating that the proteins can be used to create buffer systems for isoelectric focusing-based purification. *Oscillatoria laetevirens* NRMC-F 0139 has a large number of residues, with 18 negatively charged residues (Asp+ Glu) and 53 residues with positive charges (Arg+ Lys), compared to 20 and 34 for *Oscillatoria jasorvensis* NRMC-F 0143. According to the concentration of Cys residues, *Oscillatoria amoena* NRMC-F 0135 possesses a coefficient of extinction of 39160-38910 M^{-1} cm^{-1} at 280 nm, while *Oscillatoria jasorvensis* NRMC-F 0143 has an index of 34160-34910 M^{-1} cm^{-1}. Any protein with an instability index in excess of 40 can be considered unstable, based on the information provided by the Prot Param database (Guruprasad *et al.*, 1990). *Oscillatoria jasorvensis* NRMC-F 0143 had a higher aliphatic index of 89.47, subsequently followed by *Oscillatoria earlei* NRMC-F 0136 with an aliphatic index of 88.96, which has been proposed to enhance the thermal endurance of globular protein molecules. All strains' GRAVY scores demonstrate that the proteins will interact adequately in water. According to the results of the current study, random coil predominated among secondary structure elements like alpha helices and extended strand in *Oscillatoria acuta* NRMC-F 48, *Oscillatoria splendida* NRMC-F 0141 and *Oscillatoria cortiana* NRMC-F 83, whereas alpha helices were found to be dominated in *Oscillatoria earlei* NRMC-F 0136, *Oscillatoria laetevirens* NRMC-F 0139, *Oscillatoria jasorvensis* NRMC-F 0143, *Oscillatoria amoena* NRMC-F 0135, *Oscillatoria earlei* NRMC-F 0137 (Table 3). One of their main advantages is the ability to enhance taxonomic resolution from species-level to genus-level via molecular techniques instead of microscopic ones (Garnier *et al*., 1996).

Table 2: Primary structure analysis of C-phycocyanin gene in Oscillatoriaceae.

Phytochemical parameters	Length	Isoelectric Point (PI)	Molecular weight	-Ve Charged Residues	+Ve Charged Residues	Extinction Coefficient	Instability Index	Aliphatic Index	Gravy
Oscillatoria calcuttensis (NRMC-F 0142)	205	7.05	21610.32	18	23	20650-20400	29.04	79.17	-0.128
Oscillatoria earlei (NRMC-F 0136)	194	8.47	22728.9	10	29	13660-13410	45.42	88.96	-0.067
Oscillatoria earlei (NRMC-F 0137)	202	6.96	30007.1	25	45	23660-23410	33.05	76.59	-0.208
Oscillatoria amoena (NRMC-F 0135)	190	9.45	39808.5	18	26	39160-38910	47.06	81.96	-0.464
Oscillatoria acuta (NRMC-F 48)	186	5.45	24420.11	15	18	14650-14400	22.19	69.44	-0.286
Oscillatoria jasorvensis (NRMC-F 0143)	354	9.12	39470.18	20	34	34160-34910	43.12	89.47	-0.713
Oscillatoria laetevirens (NRMC-F 0139)	204	8.76	14370.05	18	53	19660-19410	23.67	95.96	-0.342

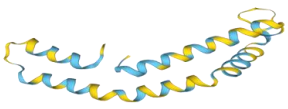
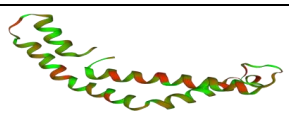
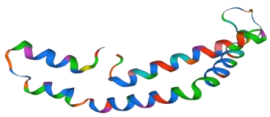
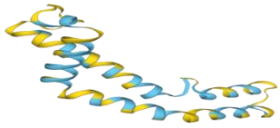
Table 3: Secondary structure analysis of C-phycocyanin gene in Oscillatoriaceae.

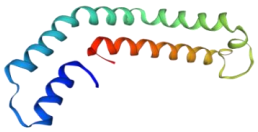
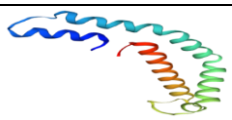
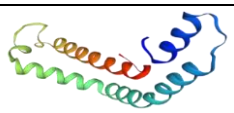
Physicochemical parameters	Sequences length	Alpha helix %	Extended strand %	Random coil %
Oscillatoria calcuttensis NRMC-F 0142	205	50.24%	11.71%	38.05%
Oscillatoria earlei NRMC-F 0136	194	54.65%	4.65%	40.70%
Oscillatoria earlei NRMC-F 0137	202	50.24%	12.68%	37.07%
Oscillatoria amoena NRMC-F 0135	190	50.49%	9.31%	40.20%
Oscillatoria acuta NRMC-F 48	186	8.09%	26.80%	70.10%
Oscillatoria jasorvensis NRMC-F 0143	354	50.49%	9.31%	40.20%
Oscillatoria laetevirens NRMC-F 0139	204	50.49%	11.76%	37.75%

SWISS homology modelling programmes were used to simulate the C-phycocyanin gene's three-dimensional structure (Arnold *et al*., 2006). According to Marti-Renom *et al.* (2000), the SWISS-MODEL system automates comparative modelling of three-dimensional protein structures and requires at least one experimentally determined template structure with a sufficient amino acid sequence resemblance to the desired sequence. SWISS- MODEL was reliant on the accuracy of the BLAST-based sequence alignment and template structure. Swiss PDB Viewer was used to make graphics and do structural analysis (Guex and Manuel 1997). This protein investigation was demonstrated by comparing 11 different Oscillatorial strains with target model reference organism *Synechococcus elongatus*, in order to determine the percentage identity (Table 4). It has been found that phycocyanin gene of 7 Oscillatorial strains have similarity more than 85% with the reference organism. Just because the protein structures are more conserved than protein sequences, noticeable instances of similarity in sequence typically include substantial structural similarity.

Comparison of predicted distribution was performed with the 2 D scatter plots such as φ; ψ pairs and are known as Ramachandran plots (Ramachandran *et al*., 1963). Rampage was used to calculate the Ramachandran plot in order to do the geometric evaluations of the modelled 3D structure. Rampage was used to analyse the torsion angles phi (φ) and psi (ψ) in order to determine the backbone configuration of the modelled structure (Lovell *et al*., 2003). For residues like Gly, Pro, Pre-Pro, and others, Rampage calculates using Phi/Psi graphs. The preferred, allowed, and outlier zones comprised the three sections of the plot. The model's plot reveals that 98% of residues fell in the favoured, 2% were allowed, and 0% was in the outlier zone (Fig. 4).

Table 4: Homology modelling of C Phycocyanin gene in Oscillatoriaceae.

Sr. No.	Strain	Model	Reference Identity (%)	Reference Organism	Reference
1.	***Oscillatoria calcuttensis*** NRMC-F 0142		89.49%	Thermophylic Cyanobacterium ***Synechococcus elongatus***	https://doi.org/10.1016/S1047-8477(02)00609-3
2.	***Oscillatoria earlei*** NRMC-F 0136		86.67%		
3.	***Oscillatoria earlei*** NRMC-F 0137		88.57%		
4.	***Oscillatoria amoena*** NRMC-F 0135		87.50%		

5.	***Oscillatoria acuta***NRMC-F 48		88.60%		
6.	***Oscillatoria jasorvensis*** NRMC-F 0143		87.71%		
7.	***Oscillatoria laetevirens*** NRMC-F 0139		85.49%		

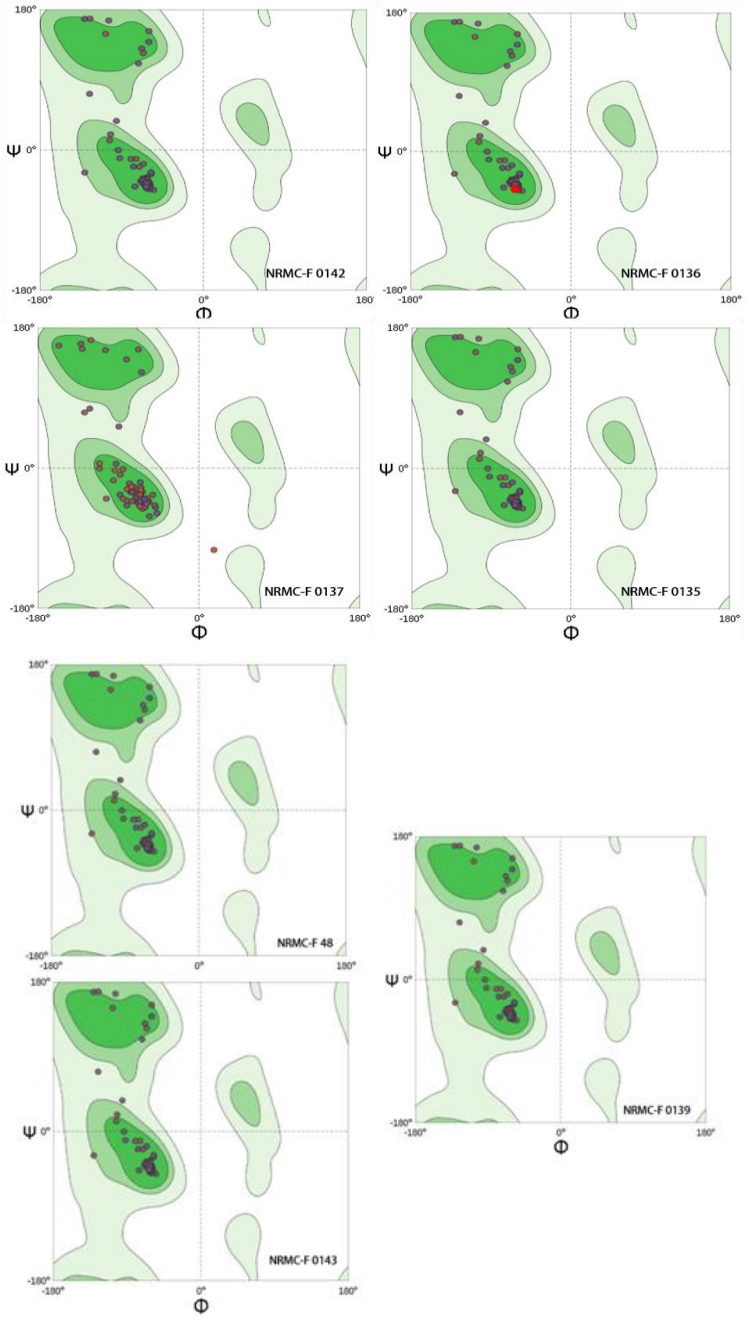


Fig. 4. Ramachandran Plot of C Phycocyanin gene in different Oscillatoriales.

CONCLUSIONS

Numerous studies demonstrated the limitations of categorising taxa based solely on morphological characteristics; therefore, phycocyanin gene sequencing was used for betterment of Cyanobacterial identification (Shakena *et al*., 2022; Heidari *et al*., 2003). Utilising the phycocyanin gene, the genetic diversity of the Oscillatoriaceae family Obtained from NRMC-F was studied. In order to expose the variability, the PC gene's sequence was further analysed using Phylogenetic analysis, primary and secondary structure prediction. The gene sequence was also compared with the reference phycocyanin gene from NCBI to determine the identity percentage among different strains belonging same genus with the help of Homology modelling.

FUTURE SCOPE

Both Phycocyanin and CYA 16S rRNA gene primer can be used as a suitable molecular marker in order to differentiate strain level variation belonging to same genus and can also act as a prominent Barcoding marker for Cyanobacteria.

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Conflict of Interest. None.

REFERENCES

Abed, R. M., Dobretsov, S. and Sudesh, K. (2009). Applications of cyanobacteria in biotechnology. *Journal of applied microbiology*, *106*(1), 1-12.

Alvarenga, D. O., Fiore, M. F. and Varani, A. M. (2017). A metagenomics approach to cyanobacterial genomics. *Frontiers in Microbiology*, *8*, 809.

Anand, N., Thajuddin, N. and Dadheech, P. K. (2019). Chapter 3 - Cyanobacterial Taxonomy: Morphometry to Molecular Studies. Cyanobacteria. From Basic Science to Applications. Pages 43-64.

Arnold, K., Bordoli, L., Kopp J. and Schwede, T. (2006). The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. *Bioinformatics*, *22*, 195-201.

Carr, N. G. and Whitton, B. A. eds., (1982). *The biology of cyanobacteria* (Vol. 19). Univ of California Press.

Desikachary, T. V. (1959). Cyanophyta, Indian council of agricultural research. New Delhi, I.C.A. Rmonographs on algae (pp. 686).

Ezhilarasi, A., Thajuddin, N. and Anand, N. (2018). Molecular taxonomic studies on cyanobacteria. In: Bioprospecting of algae (Prof. J.P. Sinha Memorial Volume) (Eds): M.N. Noor, S.K. Bhatnagar & Shashi K. Sinha. Publisher: Society for Plant Research, India 293 – 308pp.

Garcia-Pichel, F. (2009). Cyanobacteria. In *Encyclopedia of microbiology* (pp. 107-124). Elsevier Inc.

Garnier, J., Gibrat, J.F. and Robson, B. (1996). GOR secondary structure prediction method version IV, *Methods in Enzymology*, *266*, 540-553.

Geitler, L. (1932). Cyanophyceae. In L Rabenhorst's Kryptogamen Flora, Akademische Verlagsgesellschaft, Leipzig, pp. 1196

Guex, N. and Manuel, C. P. (1997). Data modelling, analysis and classification SWISSMODEL and the Swiss-Pdb Viewer: An environment for comparative protein modelling. *Electrophoresis*, *18*, 2714-2723.

Gupta, T., Singh, J., Kaur, S., Sandhu, S., Singh, and Kaur, I. P. (2020). Enhancing bioavailability and stability of curcumin using solid lipid nanoparticles (CLEN): A covenant for its effectiveness. *Frontiers in Bioengineering and Biotechnology*, *8*, 879.

Guruprasad, K., Reddy, B. V. and Pandit, M. W. (1990). Correlation between stability of a protein and its dipeptide composition: A Novel approach for predicting in vivo stability of a protein from its primary sequence. *Protein Engineering*, *4*(2), 155-161.

Heidari, F., Riahi, H., Yousefzadi, M. and Shariatmadari, Z. (2013). Morphological and phylogenetic diversity of cyanobacteria in four hot springs of Iran. *Iranian Journal of Botany*, *19*, 162–172.

Iteman, I., Rippka, R., Tandeau, D. N. and Herdman, M. (2002). rDNA analyses of planktonic heterocystous cyanobacteria, including members of the genera *Anabaenopsis* and *Cyanospira*. *Microbiology*, *148*, 481–496.

Katie Shiels, Norma Browne, Fiona Donovan, Patrick Murray and Sushanta Kumar Saha (2019). Molecular Characterization of Twenty-Five Marine Cyanobacteria Isolated from Coastal Regions of Ireland. *Biology* (Basel). 2019 Sep; *8*(3), 59.

Lovell, S. C., Davis, I. W., Arendall, W.B., De Bakker, P. I. W., Word, J. M., Prisant, M. G., Richardson, J. S. and Richardson, D. C. (2003). Structure validation by Cαgeometry: ϕ, ψ and Cβ deviation. *Proteins: Structure, Function, and Genetics*, *50*(3), 437–50.

Ludwig, W. and Klenk, H. P. (2005). Overview: A phylogenetic backbone and taxonomic framework for prokaryotic systematics. Bergey's manual® of systematic bacteriology. Pp 49–66.

Lyra, C., Suomalainen, S., Gugger, M., Vezie, C., Sundman, P., Paulin, L. and Sivonen, K. (2001). Molecular characterization of planktic cyanobacteria of *Anabaena*, *Aphanizomenon Microcystis* and *Planktothrix* genera. *International Journal of Systematic and Evolutionary Microbiology*, *51*, 513–526.

Marti-Renom, M.A., Stuart, A.C., Fiser, A., Sanchez, R., Melo, F. and Sali, A. (2000). Comparative protein structure modelling of genes and genomes. *Annual Review of Biophysics and Biomolecular Structure*, *29*, 291-325.

Muralitharan, G. and Thajuddin, N. (2011). Rapid differentiation of phenotypically and Geno-typically similar *Synechococcus* elongates strains by PCR finger printing. *Biologia*, *66*(2), 238–243.

Muralitharan, G. and Thajuddin N., (2008). Evidence on the presence of tRNAfmet group I introns in the marine cyanobacterium *Synechococcus elongatus*. *Journal of Microbiology and Biotechnology*, *18*(1), 23–27.

Neilan, B. A. (2002). The Molecular evolution and DNA profiling of toxic cyanobacteria. *Current Issues of Molecular Biology*, *4*, 1-11.

Ouellette, A. J. A. and Wilhelm, S. W. (2003). Toxic cyanobacteria: the evolving molecular toolbox. *Frontiers in Ecology and the Environment*, 1, 359-366.

Pilot, T. J. and Fox, J. L. (1984). Cloning and sequencing of the genes encoding the *α* and *β*subunits of C-phycocyanin from the cyanobacterium *Agmenellum quadruplilcatum*. *Proceedings of National Academy of Sciences*, USA. *81*, 6983–6987.

Ramachandran, G.N., Ramakrishnan, C. and Sasisekharan, V. (1963). Conformation of polypeptides and proteins. *Journal of Molecular Biology, 7*, 95–99.

Saitou, M. and Nei, M. (1987). The neighbour joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406–425.

Shakena T., Adams, S. J. and Majeed, A. (2023). Study on the Bioconversion of Curcumin to Calebin-A Using *Spirulina subsalsa* and Its Taxonomic Resolution Using 16S rRNA Analysis. *Applied Biochemistry Biotechnology*, *195*, 2933–2946.

Smith, A. A., Coomes, M. W. and Smith, T. E. (2008). Isolation and sequence of the phosphoenolpyruvate carboxylase gene of the marine cyanobacterium *Synechococcus* PCC 7002. *Journal of Biological Sciences*, *8*, 1261–1270.

Smoker, J. A. and Barnum, S. R. (1988). Rapid small-scale DNA isolation from filamentous cyanobacteria. *FEMS Microbiology Letters*, *56*, 119–122.

Whitton, B. A. and Potts, M. (2012). Introduction to the cyanobacteria. Ecology of cyanobacteria II: their diversity in space and time, pp.1-13.

Whitton, B.A. ed., (2012). Ecology of cyanobacteria II: their diversity in space and time. Springer Science & Business Media.

Yu, P., Li, J. R. and Cen, P. L. (2002). Cloning and sequencing of the phycocyanin gene from *Spirulina maxim*a and its evolutionary analysis. *Journal of Applied Phycology*, *14*, 307–310.

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