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Molecular Characterization and Phylogeny of Marine Cyanobacteria from Palk Bay Region of Tamil Nadu, India

[1]D. Pandiaraj, [1]D. Mubarak Ali, [1]R. Praveen Kumar, [2]S. Ravikumar and [1]N. Thajuddin
[1]Department of Microbiology, School of Life Science, Bharathidasan University Tiruchirapalli, India
[2]Department of Oceanography and Coastal Areas Studies, Alagappa University, Thondi Campus, Karaikudi, India

Corresponding Author: N. Thajuddin, Department of Microbiology, Bharathidasan University, Tiruchirappalli-623409, Tamil Nadu, India Tel: +91 431 2407082 Fax: +91 431 2407045

ABSTRACT

Most of the marine cyanobacteria especially to the order Chroococcales, Oscillatoriales, Nostocales occur ordinarily as planktonic forms. Their taxonomic assignment was based on morphological and cytological characteristics. The genetic variation in the species and strain level morphological and cytological features is not reliable and molecular characterization of cyanobacteria is necessary for better identification. The samples were collected from Thondi and Kattumavadi in Palk Bay region of Tamil Nadu, India. Biodiversity of cyanobacteria were documented, purified and maintained. The two strains, *Phormidium chlorinum* NTMP01 and *Jaaginema psedogeminatum* NTMP02 were selected for further molecular characterization based on 16S rDNA sequence in the strain. Evolutionary relationship and secondary structure was constructed with the sequence. The sequences were submitted to GenBank with accession numbers GU812856 and GU812857.

Key words: Biodiversity, cyanobacteria, 16S rDNA, phylogeny, gene sequence

INTRODUCTION

The cyanobacteria are morphologically distinct group of oxygenic photosynthetic organisms which inhabit both terrestrial and aquatic ecosystem which habit both terrestrial and equatic ecosystem as well as in both fresh and marine water. These cyanobacteria are classified into prokaryotic algae since they lack nuclear membrane as in bacteria (Konstantinos and Jiri, 1985). The cyanobactreria, until recently in oblivion, uncared for and unrecognized, have shot in to frame and popularity owing to a host of their innate properties that make them ideal organisms for use in a variety of ways to meet our needs and to promise us a bright future (Thajuddin and Subramanian, 2005). Besides ecological significance, they offer a great potential tool as an organisms for the biotechnological interest such as mariculture, food, feed, fuel, fertilizer, medicine and in combating pollution.

Morphological characters used to distinguish species are the presence of a sheath around individuals or around a colony, pigmentation, trichome width, cell division planes, cell shape, cell dimension and cell numbers in a colony. However, some of these characters were shown to be variable with changing environmental conditions. The cyanobacteria are found to be predominant in causing blooms on the water surface in fresh water, fish ponds (Shaaban and Sakr, 2001) and from diverse environment of acidic conditions were studied for their tolerance

mechanism (Karthikeyan and Gopalaswamy, 2009). The hypersaline cyanobacteria were also isolated from saltpans of Kattumavadi and applied for decolourization of paper mill effluent (Nagasathya and Thajuddin, 2008).

Molecular assessment of cyanobacterial biodiversity were studied in using the specific markers like 16S rDNA, phycocyanin locus, nif gene, rpo gene, ITS region, phosphoenolpyruvate carboxylase gene etc. (Smith *et al.*, 2008). A general overview of biodiversity assessment, molecular techniques and markers used for biodiversity assessment recommends combinatorial approach with different molecular markers. It is likely to improve the degree of resolution and provide as possible the broadest picture and in depth information about biodiversity documentation (Kumari *et al.*, 2009). In general, current 16S rDNA-based phylogenies are congruent with well supported branches of rbcl-based phylogenies (Rudi *et al.*, 1998). The present study focused on the 16S RNA based identification of cyanobacteria and to understand the phylogenetic relationship of isolated marine cyanobacteria.

MATERIALS AND METHODS

Sample collection: Samples were collected from Manora to Mallipattinam in Tamil nadu, India during May, 2009. Specimens were later transferred to Erlenmeyer flasks containing sea water, MN+ medium (Rippka *et al.*, 1979). They were maintained in culture room under white fluorescence lamps (1400 lux); 14±10 L/D at 25±2°C. Routine microscopic examinations of specimens were carried out regarding cell size and characteristic morphological features. Physiochemical parameters of water sample was analysed by standard methods (APHA, 1998).

DNA extraction and amplification: The extraction of genomic DNA from cyanobacterial isolates were carried out. (Smoker and Barnum, 1988). PCR amplification was performed for the purified DNA using CYA 106 (5'-CGG ACG GGT GAG TAA CGC GTGT-3') and CYA 781 (5'-GAC TAC TGG GGT ATC TAA TCC CA T-3') primers. The polymerase chain reactions conditions include initial denaturation of template DNA was achieved at 94°C for 2 min. Further denaturation was carried out at 94°C for 5 sec; annealing at 47°C for 10 sec, elongation at 72°C for 30 sec for 40 cycles and final elongation at 70°C for 7 min. Amplified products were isolated by electrophoresis on 1.2% agarose gel using 1X TAE buffer at a constant supply of 100 V for 30 min. Again, resolved bands were documented under Transilluminator. Sequencing was done with amplified samples with respective forward and reverse primers and sequences were submitted to GenBank via BankIt submission tool.

Construction of phylogeny and secondary structure prediction: The obtained nucleotide sequence was analyzed for secondary structure prediction. Gene Bee online software was used to predict secondary structure. The query sequence was uploaded in alignment box and the query was submitted to Gene Bee analyzer for structural analysis. The structure was predicted and compared with their stem and loop models. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site and it was computed in MEGA 5 software.

RESULTS AND DISCUSSION

The environmental factors which favour the growth of the cyanobacteria includes high nutrient concentration and light availability. The physic chemical parameters of water samples were

Table 1: Physiochemical parameters of collected water samples from palk bay region

Physicochemical analysis	Manora	Kattumavadi
Temperature (°C)	28.00	29.00
pH	7.50	7.50
Salinity (ppt)	30.00	29.00
Alkalinity (mg L^{-1})	120.00	130.00
Ammonia (mg L^{-1})	4.34	4.10
Chloride (mg L^{-1})	2235.00	2210.00
Nitrite (mg L^{-1})	0.68	0.75
Nitrate (mg L^{-1})	7.50	7.00
Phosphate (mg L^{-1})	3.26	3.15
Sulphate (mg L^{-1})	2541.00	2540.00
Dissolved oxygen (mg L^{-1})	6.20	6.00
Chemicaloxygendemand (mg L^{-1})	67.82	66.53
Biologicaloxygendemand (mg L^{-1})	20.00	21.00

analysed and tabulated (Table 1) to know the nature of environment of the isolated stains. In recent years, cyanobacteria have generated interest for producing valuable compounds ranging from therapeutic proteins to biofuels. Nutritional and medical application are most suitable for these organisms because many biomolecules expressed by marine cyanobacteria.

Totally sixteen marine cyanobacterial strains were recorded from two places in Palk Bay region and based on their morphology they were identified (Fig. 1a-l) as *Chroococcus minor, Spirulina subsalsa, Spirulina princeps, Oscillatoria subbrevis, Oscillatoria salina, Oscillatoria boryana, Oscillatoria limosa, Oscillatoria* sp., *Phormidium chlorinum, Jaaginema pseudogeminatum, Oscillatoria formosa, Anabaena* sp., *Phormidium* sp., *Pseudoanabaena* sp., in the study area. The isolates of *P. chlorium* NTMP01 and *J. pseudogeminatum* NTMP02 were subjected to the molecular characterization. Currently there is an increased interest in molecular techniques to resolve many of the issues and problems in the taxonomy of cyanobacteria. Jarousha (2002) have reported that higher diversity of the blue-green algae may be attributed to high nitrate values during the rainy season.

Among the most popular molecular techniques, the sequence determination of small subunit ribonucleic acids is widely employed. Vandamme *et al.* (1996) reported that the genetic constitution of the cyanobacteria contributes significantly to the revision of their taxonomy and relevant classification reflects the phylogenetic relationships. The integration of phenotypic, genotypic and phylogenetic information renders possible a consensus type of taxonomy known as polyphasic taxonomy. Ibraheem and Al-Sherif (2009) reported that the flowering plants and algal taxa were controlled by the edaphic factors and physico-chemical characters of the soil.

The development of a molecular based identification for marine cyanobacteria is essential for the taxonomy. The genomic DNA of *Phormidium chloronum* NTMP01 and *Jaaginema pseudogeminatum* were extracted (Fig. 2) and the genomic DNA was electro eluted in the agarose gel. Approximately 750 bp of amplified locus of 16S rDNA was observed (Fig. 3). The obtained sequences of 16S rDNA of the two isolates (*Phormidium chlorinum* NTMP01 and *Jaaginema pseudogemenatum* NTMP02) were deposited in GenBank with accession numbers GU812856 and GU812857. Wilmotte (1994) stated that a molecular approach to the systematic of cyanobacteria may be most fruitful for inferring phylogenetic relationships. Macromolecules, such as nucleic acids

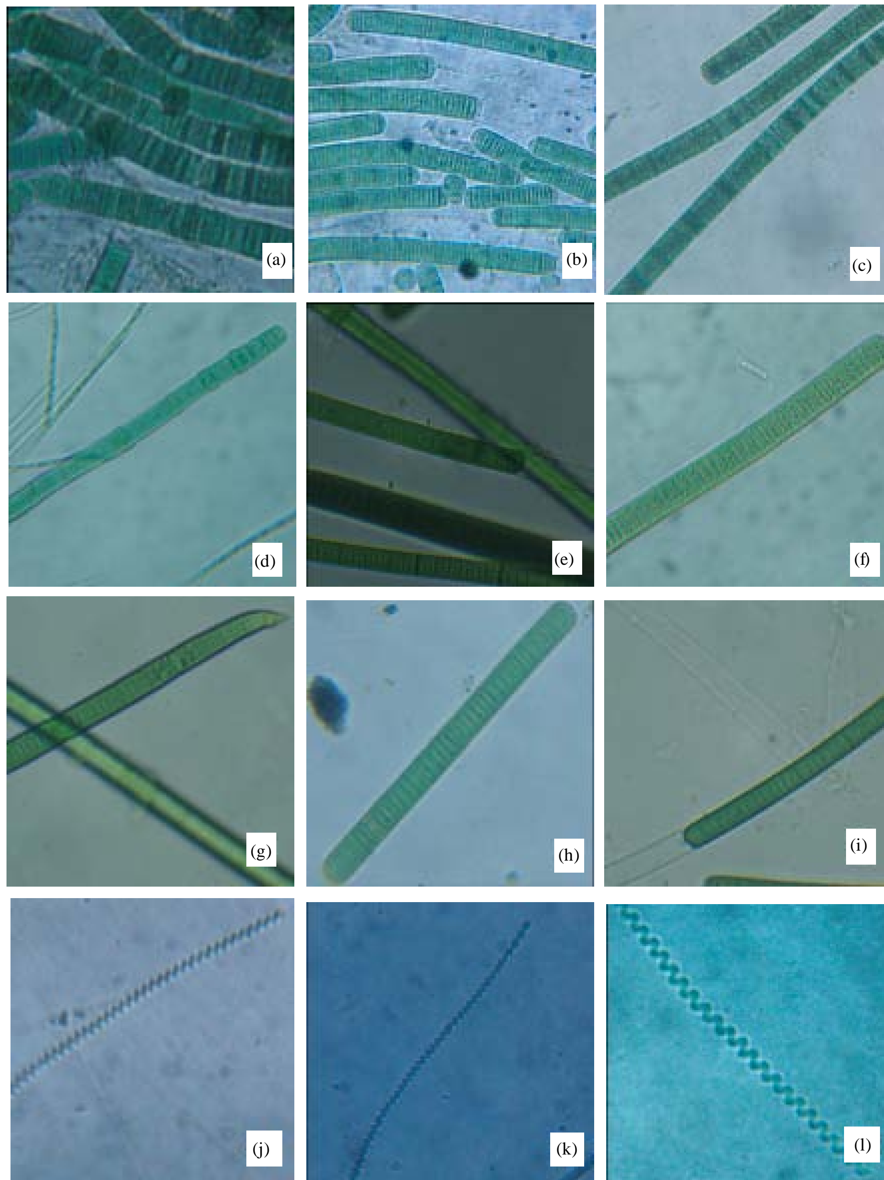

Fig. 1: Microphotograph of cyanobacterial biodiversity: (a) *Oscillatoria* sp., (b) *Oscillatoria limnsa*, (c) *Phormidium chlorinum*, (d) *Oscillatoria* sp., (e) *Jaaginema pseudogerminatum*, (f) *Oscillatoria* sp. (g) *Oscillatoria salina*, (h) *Oscillatoria princeps*, (i) *Phormidium* sp., (j) *Spirulina subsalsa*, (k) *Anabaena* sp. and (l) *Spirulina princeps*

and proteins, are copies or translations of genetic information. The methods applied involve direct studies of the relevant macromolecules by sequencing or indirectly by electrophoresis, hybridization or immunological procedures.

The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973). The optimal tree with the sum of branch length = 0.15950263 is shown in Fig. 4. The tree is drawn

Fig. 2: Genomic DNA of isolated cyanobacteria: (a) *Phormidium chlorinum* and (b) *Jaaginema pseudogeminatum*

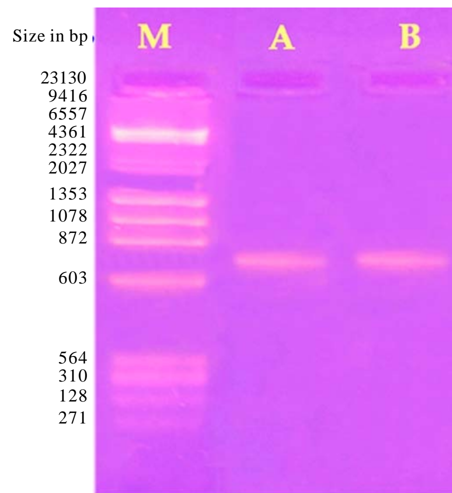

Fig. 3: 16S rDNA gene amplification: A) *Phormidium chlorinum* and *B) Jaaginema pseudogeminatum* M) 100kb marker

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 Maximum Composite Likelihood method (Tamura *et al*., 2004). All positions containing gaps and missing data were eliminated. There were a total of 301 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al*., 2007).

The secondary structure of 16S rDNA of *Phormidium chlorinum* NTMP01 showed 13 stems in their structure (Fig. 5a). Whereas, in *Jaaginema psedojeminatum* NTMP02 showed 26 stems

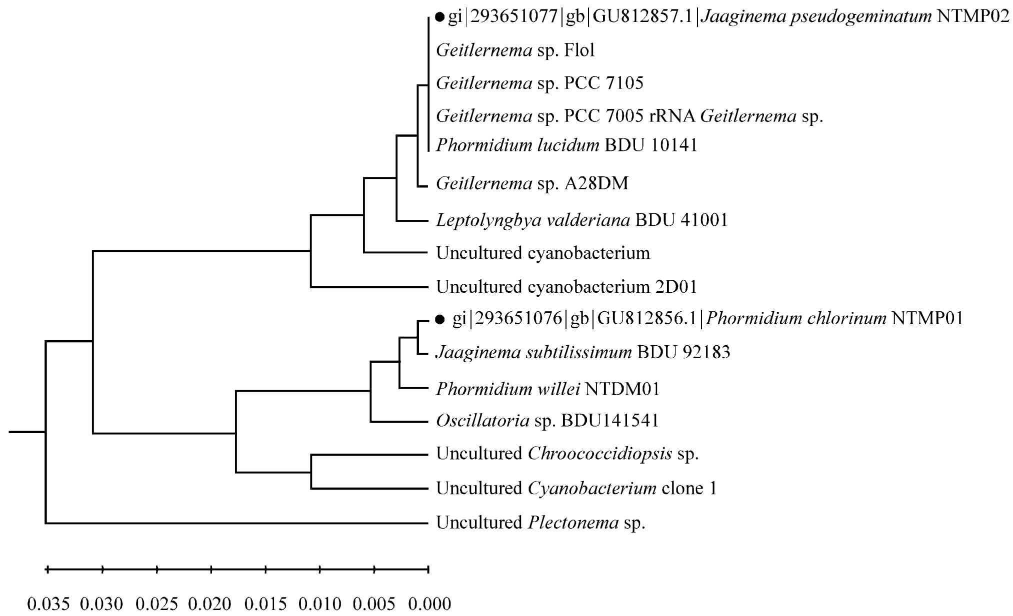

Fig. 4: Evolutionary relationships of cyanobacteria, *Phormidium chlorinum* (GU812856) and *Jaagnima pseudogeminatum* (GU812857)

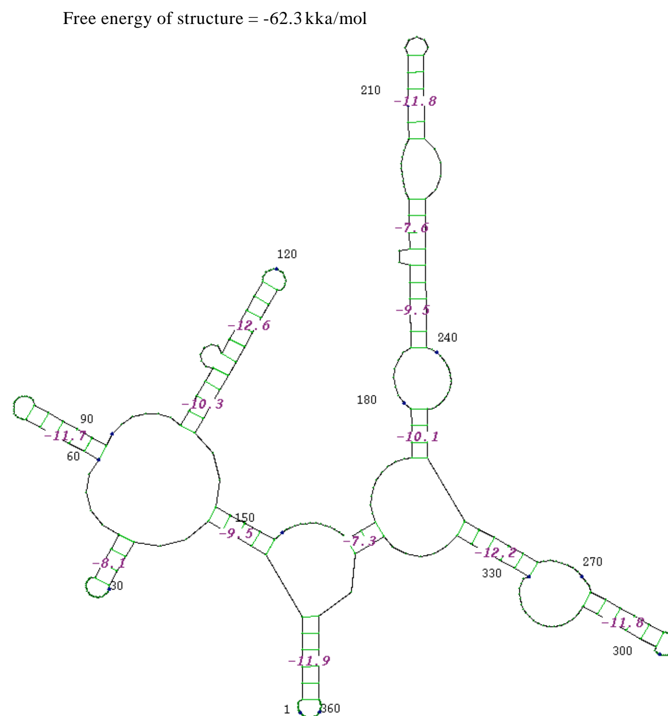

Fig. 5a: Secondary structure of *Phormidium chlorinum* NTMP01 with stem and loop structure

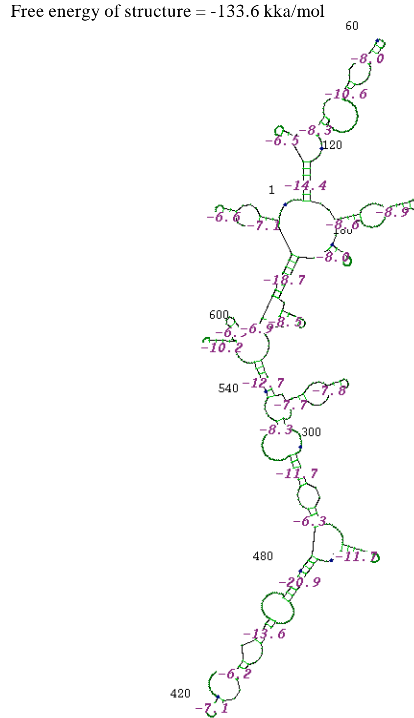

Fig. 5b: Secondary structure of *Jaagienema pseudogeminatum* NTMP01 with stem and loop structure

(Fig. 5b). In their structure, however, these isolates were differed in their energy threshold, cluster factor, conserved factor, compensated factor, conservatively, part of sequence, greedy parameter and treated sequence as indicated by Genbee software (http:/www.genebee.nsu.su).

CONCLUSION

The cyanobacteria are distributed in the wide range of ecosystems from terrestrial to marine. This work emphasizes on the moleculat characterization and phylogeny of marine cyanobacteria based on the 16S rDNA gene sequences. The water parameter was also analysed for the monitoring the biodiversity in the environment. The molecular characterization is rapid and accurate technique to identify the organisms in subspecies level.

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