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Biodiversity and Molecular Evolution of Microalgae on Different Epiphytes and Substrates

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Abstract: An exploration of the microalgal biodiversity from different epiphytes and substrates of pool water in temple at Tiruchirappalli District was studied. Totally ten epiphytic forms were selected for this investigation. In that, totally 44 species of 30 genera belonging to 3 families of the Chlorophyceae, Cyanophyceae (heterocystous and non-heterocystous) and Bacillariophyceae were identified and recorded. The dominant species in this environment were Cyanobacteria (*Chroococcus* sp. and *Oscillatoria* sp., *Phormidium* sp.), Green algae (*Tetradron* sp. and *Scenedesmus* sp.) and Diatom (*Fragilaria* sp. and *Navicula* sp.) were documented. The molecular taxonomy of cyanobacteria were also analyzed, in this regards, DNA was extracted; 16S rDNA gene was amplified and sequenced. The evolutionary relationship was found in the epiphytic microalgae by Neighbour-Joining method by construction of phylogenetic tree.

Key words: Biodiversity, microalgae, cyanobacteria, 16S rDNA, phylogeny

INTRODUCTION

Microalgae are photoautotrophic oxygen evolving microorganisms which includes (green algae, cyanobacteria and diatoms) often inhabit various types of substrate, especially light and water resources and reservoirs. In addition to their morphological diversity and extensive distribution, microalgae reflect a wide range of physiological properties and are more tolerant to environmental stress (De Marsac and Houmard, 1993). Microalgae are reported to be one of the principal component involved in biofilm formation and are responsible for several problems in many industrial cooling systems (Ludyansky, 1991; Callow, 1993). In most of the environment, microalgae are the primary producer at the base of the food web of the ecosystem and moreover, these are symbionts of the variety of other organisms, viz. the marine diatom *Rhizosolenia*, leaves of *Azolla* and the root of *Cycas* (Thajuddin and Subramanian, 2005). Some algal forms can be useful indicator on which major water management practice, pollution studies and water quality analysis (Pandey *et al*., 1998). The nature of production, distribution and relationship of the phytoplankton and zooplankton vary with the prevailing environmental conditions. The major freshwater phytoplankton groups of green algae, diatoms and cyanobacteria. In this investigation, we studied the biodiversity of different substrate inhabiting microalgae in pool water in Tiruchirappalli, Tamil Nadu (India) and 16S rDNA based molecular phylogeny of isolated cyanobacteria.

MATERIALS AND METHODS

Sample collection: Collections were made from various epiphytic or substrate in pool water of the Parthasarathy temple at Tiruchirappalli, Tamil Nadu, India. Ten different epiphytic and substrate were selected for microalgal samples collection. The methods used for the collection and studies of the same as described previously (Anand, 1998). The microalgal samples were scrapped from the substrate such as Tin (1), Plastic cup (2), plastic paper (3), mango seed (4), ball (5), coconut (6), coins (7), cane cap (8), thermo coal (9) and pool water (10) were transferred into BG 11 medium which contains both N^+ and N^-nutrient medium (Fig. 1). The physiochemical parameter of water sample was analyzed by standard methods.

Biodiversity and morphological identification of microalgae: The specimens were taxonomically

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Fig. 1(a-i): Distribution of microalgae in different epiphytes and substrates, (a) Tin, (b) Plastic cup, (c) Plastic paper, (d) Mango seed, (e) Ball, (f) Coconut shell, (g) Coins, (h) Thermo coal and (i) Cane cap

Table 1: Primers for the amplification of the cyanobacterial 16S rDNA gene

Primer name	Sequence 5'→3'	Target site	Reference
16S rDNA			
CYA 106F	CGG ACG GGT CAG TAA CGC GTG A	106-127	Nubel *et al.* (1997)
CYA 781R(a)	GAC TAC TGG GGT ATC TAA TCC CAT T	781-805	

determined with the help of standard literatures i.e., Desikachary (1959) for cyanobacteria; Desikachary *et al.* (1987) for bacillariophytes and Belcher and Swale (1978) and Hortobagyi (1973); for chlorophytes and bacillariophytes. The sizes were measured and microphotography is documented using Light Microscopy (Model OPTIKA).

Molecular analysis: In DNA extraction, the genomic DNA was extracted from the *Phormidium* and *Oscillatoria* sp., using the method of Smoker and Barnum (1988). The extracted genomic DNA was electrophoresed on 0.8% agarose against Tris-Acetic acid buffer (pH. 8.2).

In Polymerase Chain Reaction (PCR), amplification of 16s rRNA gene from *Phormidium animale* and *Oscillatoria acuminata* were made using the specific forward primer (CYA106) and the specific reverse primer (CYA781R). Primer sequences and target regions within the 16S rRNA gene are listed in Table 1. In this reaction,

50 μL of reaction volume contains 25 μL of premix (company), 1 μL of forward primer, 1 μL of reverse primer, 50 ng in 1 μL, of genomic DNA, sterile double distilled water 17 μL. The PCR procedure consisted of denaturation at 93°C for 5 min followed by 35 cycles of the following: denaturation at 93°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 3 min and final extension was 72°C for 8 min. PCR products were electrophoresised on 1.2% agarose against Tris-Acetic acid buffer (pH. 8.2). The amplified products were visualized in Uv-transilluminator.

Construction of phylogeny: The Sequencing was done using automatic sequencer. The products were sequenced in both 5'-3' direction and 3'-5' directions. The evolutionary relationship was computed using neighbor-joining method (Tamura *et al*., 2004) and is in the units of the number of base substitutions per site and it was computed in MEGA 5 software. The 16S rDNA gene sequences were analyzed with BLAST tool and submitted to GenBank (www.ncbi.nlm.nih.gov/genbank).

RESULTS AND DISCUSSION

In this present investigation, 44 species of microalgae has been distributed in ten different epiphytes were recorded and tabulated (Table 2). There are different morphological microalgae were documented, they are

Table 2: Biodiversity of Microalgae (Chlorophyceae, Bacillariophyceae and Cyanophyceae) from different epiphytes

Microalgal biodiversity	1	2	3	4	5	6	7	8	9	10
Chlorophyceae										
Chlorella vulgaris	+	+	+	+	+	+	+	+	+	+
Chlorella sp.	+	+		+	+	+	-	+	+	+
Scenedesmus dimorphus	-	-	-	+	-	-	-	-	-	+
Astrococcus sp.	-	-	-	-	-	-	-	-	-	+
Scenedesmus bicellularius	-	-	-	+	-	-	-	-	-	+
Scenedesmus sp.	-	-	-	+	-	+	-	-	-	+
Scenedesmus acuminatus	-	-	-	-	-	+	-	-	-	+
Cosmarium granatum	-	-	-	+	-	+	-	-	-	+
Cosmarium sp.	-	-	-	+	-	+	-	-	-	+
Chlorococcum humicola	+	+	-	+	-	+	-	-	+	+
Chlorococcum sp.	-	+	-	+	-	-	-	-	+	+
Pediastrum simplex	-	-	+	-	-	+	-	-	-	+
Coalastrum microsporum	-	-	-	+	-	+	-	-	+	+
Coalastrum sp.	-	-	-	-	-	-	-	-	-	+
Zygnema sp.	-	-	-	+	-	+	-	-	+	-
Tetradron minimum	-	+	-	-	-	-	-	-	-	+
Oocystis sp.	-	-	-	-	-	-	-	-	-	+
Cyanophyceae										
Anabaenopsis sp.	-	+	+	-	-	-	-	-	-	-
Pseudoanabaena sp.	+	+	-	-	-	-	-	-	-	+
Nostoc commune	-	-	-	-	-	-	-	-	-	+
Nostoc sp.	-	-	-	-	-	-	-	+	-	+
Oscillatoria acuminata	+	-	-	+	-	+	-	-	-	+
Oscillatoria animale	-	-	-	+	-	+	-	-	-	-
Oscillatoria boryana	-	-	-	+	-	+	-	-	-	-
Oscillatoria earlei	+	-	-	-	-	+	-	-	-	+
Phormidium animale.	+	+	+	-	+	-	-	-	+	+
Phormidium willei	-	-	-	-	-	+	-	-	+	+
Phormidium sp.	+	-	+	-	+	-	-	+	+	+
Calothrix branni	-	-	-	+	-	-	-	-	-	+
Merismopedia aeroginosa	-	-	-	-	-	-	-	-	-	+
Gloecapsa ovale	-	-	-	-	-	-	-	-	-	+
Gleocapsa sp.	-	-	-	-	-	-	-	-	-	+
Scytonema sp.	-	-	-	+	-	+	-	-	+	-
Synechococcus elongatus	+	-	+	-	-	+	-	-	-	+
Chroococcus minor	-	+	-	-	+	+	-	-	-	+
Bacillariophyceae										
Navicula minima	-	-	+	-	-	-	-	-	-	+
Navicula sp.	-	-	-	+	-	+	-	-	-	+
Nitzchia amphibia	-	-	-	+	-	-	-	-	-	+
Fragilaria sp.	-	-	-	-	-	-	-	-	-	+
Amphora ovale	-	-	-	+	-	-	-	-	-	+
Staruroneis sp.	-	-	-	+	-	-	-	-	-	+
Cymbella sp.	-	-	-	+	-	+	-	-	-	+
Gomphonema sp.	-	-	-	-	-	-	-	-	-	+
Euglenophyceae										
Euglena sp.	-	-	-	-	-	-	-	-	-	+

Fig. 2: Microphotograph of *Euglena* sp.

Cosmarium granatum, *Chlorococcum humicola*, *Pediastrum simplex*, *Scenedesmus dimorphus*, *Coelastrum microsporum*, *Tetraedron minimum*, *Euglena* sp. (Fig. 2) in green algae (Fig. 3); *Chroococcus* sp., *Merismopedia* sp., *Oscillatoria* sp., *Anabaenopsis* sp., *Pseudoanabaena* sp. in cyanobacteria (Fig. 4) and *Navicula* sp., *Nitzschia amphibia*, *Amphora ovalis* in diatoms (Fig. 5). There are certain members of cyanophyceae which are tolerant to organic pollution and resist environment stress caused by the pollutant. Such species can be used as Marker species or indicator of particular habitat (Prasad and Saxena, 1980).

Some microalgae such as *Chlorella* sp., *Nitzschia* sp. and *Chroococcus* sp., adhered to hard substrates (Sekar *et al*., 2004). The physiochemical parameters of the water were analyzed and tabulated (Table 3). The frequency of the some species and their survival may be due to the micronutrients present in the water, the physiochemical changes in the environment may affect particular species and induce the growth and abundance of other species (Muthukumar *et al*., 2007). While studying the bacterial attachment to surfaces (Wrangstadh *et al*., 1996) found that the higher attachment on hydrophobic surfaces is mediated by the water exclusion mechanism, whereas hydrophilic substrata water is poorly excluded resulting in less attachment (Burchard *et al*., 1990). In this study, the increased attachment observed by all the microalgae both on hydrophilic and hydrophobic substrates (tin, plastic paper and thermo coal) may be due to water exclusion mechanisms.

Table 3: Physiochemical analysis of water samples collected from temple pool water at Tiruchirappalli

Parameters	Temple pool water
Colour	Pale green
pH	8.5±0.2
Dissolved oxygen (mg L^{-1})	0.91
Ammonia (mg L^{-1})	0.5
Chloride (mg L^{-1})	1.046
Nitrate (mg L^{-1})	1.0
Nitrite (mg L^{-1})	15
Inorganic phosphate (mg L^{-1})	0.01
Alkalinity (mg L^{-1})	510
Sulphate (mg L^{-1})	5.9
Calcium	2.15

It has been reported the higher level of sulphide content is toxicity to the heterocystous forms. It has been reported that the high values of BOD, COD, Phosphates and nitrates with very DO favored the growth of cyanobacteria than any other algae (Singh and Saxena, 1969; Venkateshwarlu, 1976). The continuous discharge of oils, flowers, rice and dhal to the water may increase the concentration of sulphur and nitrogen. Increased amount of nitrogen in the water may decrease the level of heterocystous forms. Even though some microalgae inhabit unexpected substrates such as thermo coal and coins, they might have derived its nutrients from these or adapted to that environment for its survival.

It has been studied that the value of pH is at its lowest early in the morning then it rises, at night it decreases again; it was daytime fluctuation is considerable. This daily fluctuation of the pH value, in extreme cases, may even exceed 3 pH units (Hortobagyi, 1973). Some of the aerophytic algae inhabit stony substrate (Hoffmann and Darienko, 2005). It is well known that the molecular sequencing studies was confirmative procedure in the current molecular taxonomy for species level identification, 16s rRNA gene sequencing is a reliable methods for the identification of species. It has been reported that the sequence analysis of genes encoding small subunit ribosomal RNA is currently the most promising approach for the phylogenetic classification of cyanobacteria (Wilmotte, 1994). There are many workers have done with cyanobacterial gene sequencing for identification, there were critical identification methods from sponges associated cyanobacteria (Steindler *et al*., 2005), hot-spring inhabiting cyanobacteria (Weller *et al*., 1991). PCR primer is very important role for the amplification of target site; there is need specificity to target sites for PCR with absolute primers. The alternative forward primer CYA106F matches a numbers of published 16S rRNA sequences from prokaryotes with various phylogenetic affiliations outside the phylum of the cyanobacteria and also the amplification specificity checked by DGGE to investigate

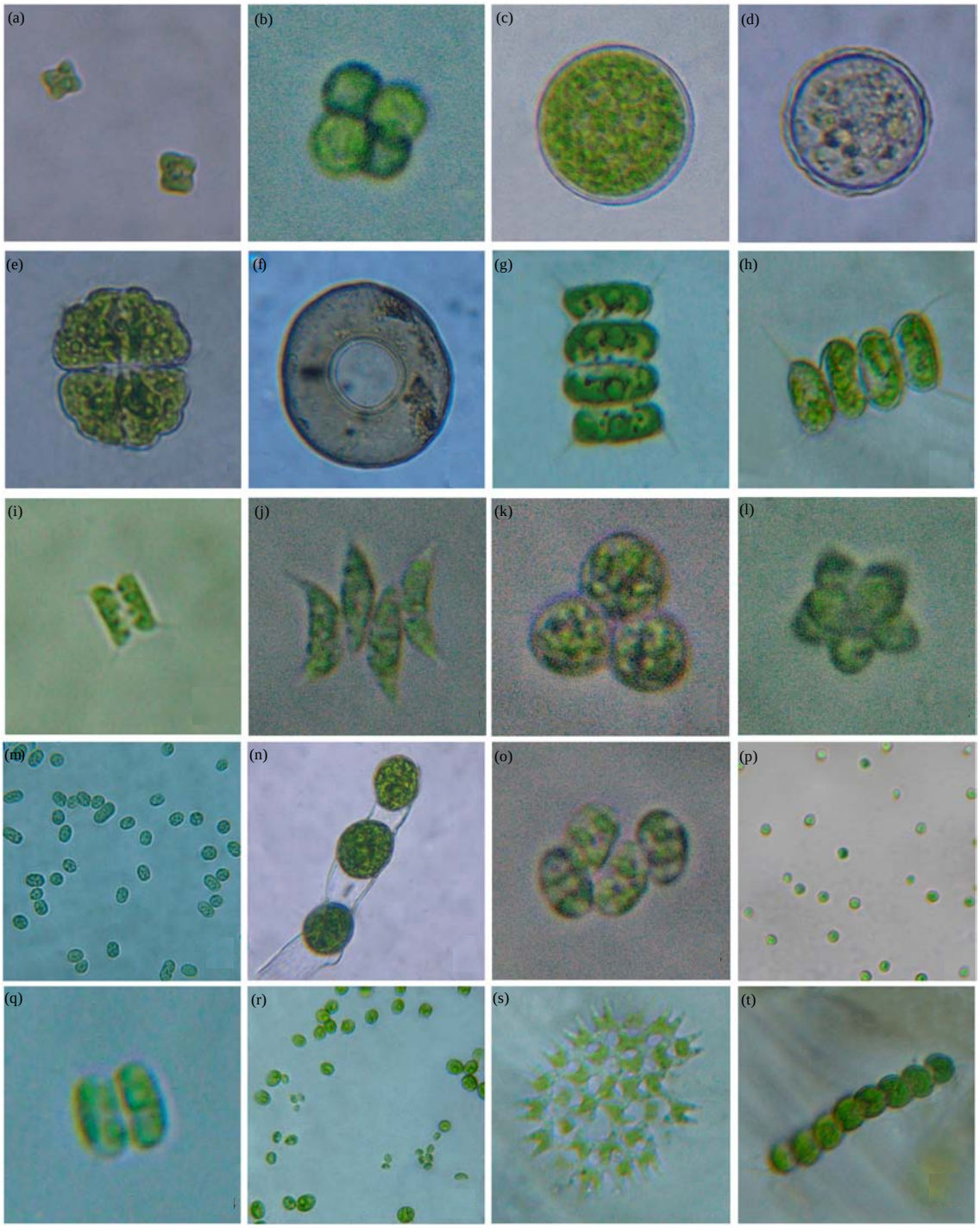

Fig. 3(a-t): Microphotograph of (a) *Tetrdron* sp., (b) *Cosmarium* sp., (c) *Chlorella* sp., (d) *Chlorella* sp., (e) *Cosmarium* sp., (f) *Oocystis* sp., (g) *Scenedesmus* sp., (h) *Scenedesmus* sp., (i) *Scenedesmus aureus*, (j) *Scenedesmus* sp., (k) *Astrococcus* sp., (l): *Coalestrum* sp., (m) *Scenedesmus* sp., (n) *Scenedesmus bijugatus*., (o) *Chlorococcum humicola*., (p) *Chlorococcum* sp., (q-r) *Zygenema* sp., (s) *Pediastrum boyana*., (t) *Scenedesmus* sp.

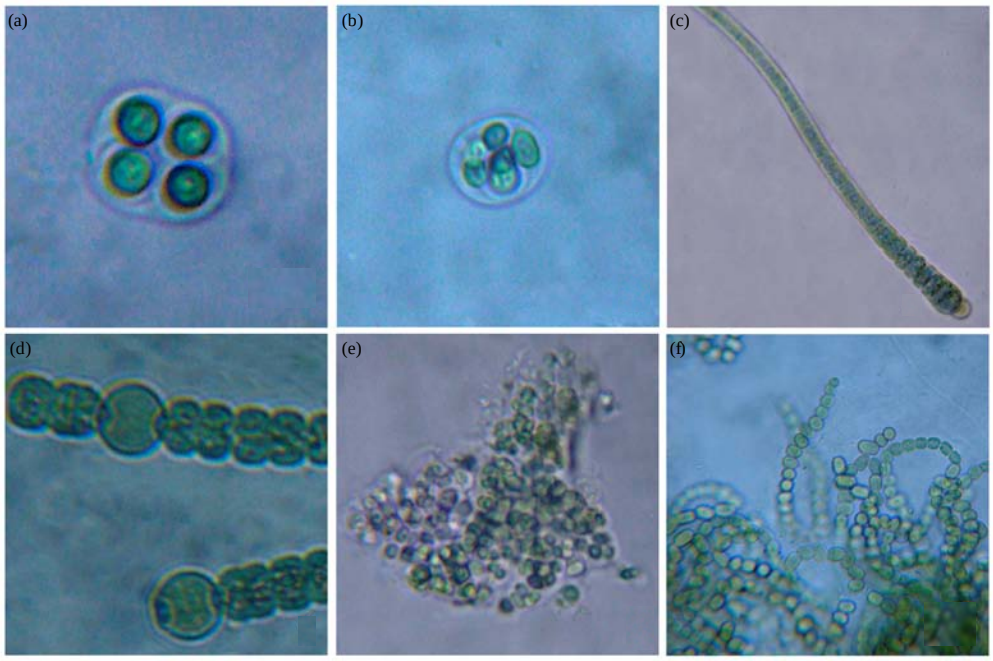

Fig. 4(a-f): Microphorgraph of (a) *Gleocapsa ovale*, (b) *Gleocapsa* sp., (c) *Calothrix braunii*, (d) *Anabaena* sp., (e) *Merismopedia* sp., (f) *Nostoc* sp.

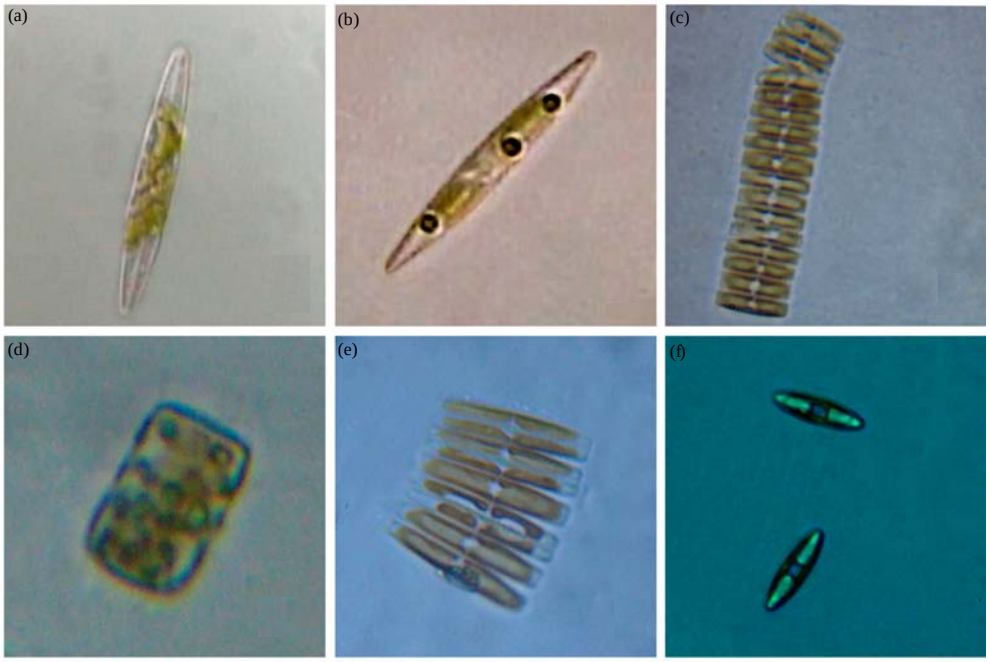

Fig. 5(a-f): Microphotograph of (a) *Navicula* sp., (b) *Nitzchia* sp., (c) *Fragilaria* sp., (d-e) *Fragilaria* sp., (f) *Stauronesis* sp.

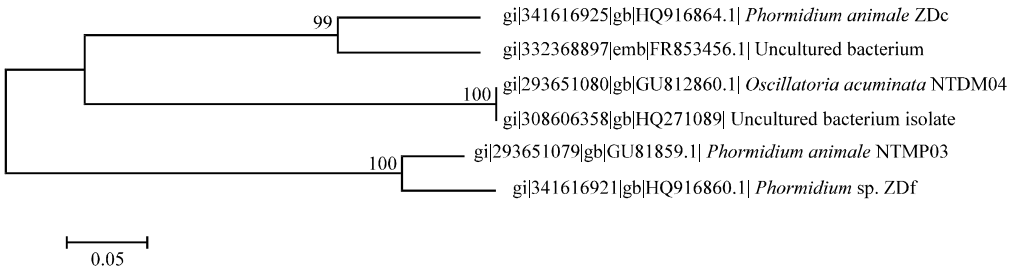

Fig. 6: Evolutionary relationship of cyanobacterium, *Oscillatoria acuminata* NTDM04 and *Phormidium animale* NTMP03

homogenecity of the sequence of PCR products prior to sequence analysis (Nubel *et al*., 1997). The genetic analysis can also be compared with hydrobiological and hydrochemical analyses and the genetic abundance profiles may provide a foundation for separating and quantifying genetically distinct groups of cyanobacteria in their natural habitats (Rudi *et al*., 2000) (Fig. 6).

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 construction of the cyanobacteria contributes significantly to the revision of their taxonomy and relevant classification reflects the phylogenetic relationships. The integration of the phenotypic, genotypic 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 were controlled by the edaphic factors and physico-chemical characters of the soil. Pandiaraj *et al.* (2012) also reported that the molecular characterization and phylogeny of marine cyanobacteria using 16S rDNA sequencing. It was well characterizing method to understand the taxonomical position of the isolates.

In this investigation micro algal biodiversity in the temple at Tiruchirappalli showed total of 44 species of 30 genera belonging to 3 families of the Chlorophyceae, Cyanophyceae and Bacillariophyceae, various types of species. Many works has been reported that the cyanobacterial flora in temples in which walls, water bodies and drainages. This may be the new report that the microalgal flora in different epiphytes in the temple water pool. Representative nucleotide sequences such as *Oscillatoria acuminata* and *Phormidium animale* have also been submitted to GenBank under the following accession numbers: GU812859 and GU812860.

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