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Diversity and RAPD finger printing of cyanobacteria from fresh water habitat of Pudukkottai, Tamil Nadu

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ABSTRACT

A great deal of research in the biogeography of marine and fresh water cyanobacteria has been carried out, but many unknown persist, and more work is needed to elucidate and understand thick complexity. The present investigation aimed at analyzing their distribution, generic diversity and abundance in selected ecologies such as river, ponds and rainfall of Melapponnanviduthy at Pudukkottai district. The physico – chemical analysis of water showed variations. Small pieces of microbial colonies of fresh water cyanobacterial isolates were picked out from the BG-11 medium for laboratory analysis. Totally ten genera were identified in the study. The selected cyanobacterial cultures were subjected to molecular characterization by using RAPD – PCR. Then the Phenogram generated revealed two clusters A and B. The cluster A was further divided into two sub clusters A_1 and A_2. The sub cluster A consist of two strains (Phormidium lucidum ***SG01*** *and P. lucidum* ***SG02),*** *and the cluster B having only one strain (Phormidium lucidum* ***SG03).*** *From the banding pattern, it was clearly shown that all the strains tested were different from each other.*

Key words: Cyanobacterial cultures, physico – chemical analysis, RAPD – PCR, Phenogram.

INTRODUCTION

A great deal of research in the biogeography of marine and fresh water cyanobacteria has been carried out, but many unknown persist, and more work is needed to elucidate and understand thick complexity. It is now known that these organisms live in every corner of the ocean. Their habitats are diverse and include open water, sediment, bodies of marine micro organisms, estuaries, and hydrothermal events. By studying their habitats, scientists have developed a limited ability to predict the composition of aquatic microbial communities. Tropical conditions

such as those in India provide favourable environment for the luxuriant growth of these organisms in the natural ecosystems such as different types of soil, freshwater bodies, oceans, saline backwaters, estuaries and also hyper saline saltpans.[7,20&2].

Algae are found in soil as well as water (fresh and marine). Algae are a group of autotrophic organisms that synthesized there food by photosynthesis. Algae are a rich source of carbohydrates, protein, enzymes, fiber, vitamins and minerals etc. [10] . The physico-chemical characteristics, like dissolved oxygen, salinity, pH, temperature and electrical conductivity diffused from month to month and layer to layer. All the factors except dissolved carbondioxide, exhibited positive correlations in the each other.

Development of modern techniques, especially PCR had made it possible to compare cyanobacterial isolates more carefully and critically. Large number of DNA based methods, like RAPD and related DNA finger printing techniques have been used to classify and identify their organisms [21 & 22]. The randomly amplified poly morphic DNA (RAPD) technique, in conjunction with PCR has been employed to identify many organisms to the strain level of classification.

MATERIALS AND METHODS

Sample Collection

In the present study, the cyanobacterial mats were collected randomly from five different fresh water habitat of Melapponnanviduthy Village at Pudukottai, during Dec'2006 – Jan' 2008. At each place 1 – 4 samples were collected in the sterile polythene bags. The samples were transported to laboratory and analyzed immediately. The collected cyanobacterial samples were transferred to conical flasks with BG11 N^+ and BG11 N^- medium. [13]. Cyanobacterial species were identified using the publication of [5,3 & 19]. Photomicrography was taken using Nickon photomicrographic Unit (Japan).

Isolation and Extraction of Chromosomal DNA from Cyanobacteria [19]

Procedure:

A 1-ml aliquot of mid to late-log-phase culture was centrifuged at 14,000 rpm for 5 minutes. The medium was decanted and the pellet was resuspended in 500µl of 50 mM Tris-Hcl (pH 8.0)-5 mM EDTA (pH 8.0)-50mM Nacl. Lysozyme was added to obtain a final concentration of 1mg/ml, and the solution was incubated at 55°C for 30 minutes. After the addition of 10µl of proteinase K (10mg/ml) and 20µl of 10% sodium dodecyl sulfate, the mixture was incubated at 55°C for 10 minutes or until the solution cleared (complete cell lysis). The solution was chilled on ice and extracted with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1). The organic extraction was repeated, and the supernatant was added to an equal volume of 4M ammonium acetate. Total genomic DNA was precipitated by the addition of 2 volumes of isopropopanol followed by centrifugation of 14,000 rpm at room temperature. The supernatant decanted, pellet washed with 70% ethanol. The pellet was vacuum dried and resuspended in 200µl of Tris EDTA buffer.

PCR Amplification [6]

All the PCRs were carried out in a 50µl volume as per the instruction by the manufacturer (Finzymes, Finland)

10x PCR buffer	-	5 µl
dNTP mix	-	1 µl
Taq DNA polymerase	-	0.5 µl
100 p moles of Primers	-	2 µl
Template DNA (50ng)	-	1 µl
Sterile Double distilled water	-	40.5 µl

The DNA amplification was performed in LongGene MG 25+, with the following program.

Initial Denaturation	:	94 ° C for 4 minutes
Denaturation	:	92 ° C for 1 minute
Annealing	:	45 ° C for 1 minute
Extension	:	72 ° C for 1 minute
Final Extension	:	72 ° C for 5 minutes
Number of cycles	:	30

Electrophoresis [7]

After the reaction, amplified PCR products were subjected to 1.2% agarose gel electrophoresis, using 1x Tris- borate – EDTA buffer and electrophoresed 3 hours at 50V. The gels were stained in an ethidium bromide solution (0.5mg/ml) to Visualize RAPD bands and documented using bioview Gel Documentation System.

Calculation of genetic distance with RAPD markers

Each band visualized on a gel was considered RAPD marker and part of the total RAPD fingerprint generated for a species of cyanobacteria. The presence or absence of a band at any position on the gel was used to construct a binary matrix for cyanobacterial RAPD markers from the described reactions. Genetic distances between species were calculated by using the algorithm of [9], as provided in the RAPD distance software package developed by [1]. A pair wise comparison of genetic distance for all cyanobacterial patterns was used to create a phenogram based on the Neighbor Joining method and the program NJ Tree [14].

RESULTS AND DISCUSSION

The present study aimed at analyzing their distribution, generic diversity and abundance in selected ecologies such as river, ponds and rainfall of Melapponnanviduthy at Pudukkottai district (Table - 1). Totally ten genus were identified in all the stations. Among the ten genera *Phormidium* was the most abundance genera. The classical ecological approach involving characterization of the community structure by identification and enumeration of the species present, followed by assigning roles in ecosystem function to species or groups was employed [12]. The water ecosystem represents a favorable environment for the growth of cyanobacteria fulfilling the requirements of the growth of light, temperature and nutrient availability in optimal manner. According to the physico – chemical parameters, the cyanobacterial diversity may be altered. The salient features of water sampling ecologies surveyed, in the present study are given along with their electrical conductivity, dissolved oxygen and pH values. The water samples collected from all station exhibited as pH range was 7.07 – 7.5 (Table – 2).

Morphologically similar but genetically distinct cyanobacteria may coexist in one body of water and form separate blooms, or there may be a mixture of cyanobacteria within one bloom (e.g., *Nodularia, Synechococcus, Microcystis* and *Cylindrospermopsis* [15]). A genetically mixed population, such as those examined from the Florida lakes, may be the result of accelerated molecular evolution in highly favorable environmental conditions (warm temperatures, abundant nutrients, and sufficient light year-round) or the more recent introduction of new strains.

Table 1. Genera wise abundance and diversity in the various locations

S. No	Genus	Melapponnan Viduthy (S1)	Malayur (S2)	Ponnanviduthy (S3)	Alangudi (S4)	Thiruvarankulam (S5)
1	*Phormidium*	+	+	+	+	+
2	*Oscillatoria*	+	-	+	+	+
3	*Lyngbya*	+	+	+	+	-
4	*Leptolyngbya*	-	+	-	-	-
5	*Chroococcus*	-	-	+	-	+
6	*Aphanocapsa*	+	-	+	-	+
7	*Microcystis*	-	-	+	+	-
8	*Synecoccus*	+	-	-	-	-
9	*Nostoc*	-	+	+	-	-
10	*Calothrix*	-	+	+	-	-

Present - +
Absent - -
Collection Station – S1 – S5

Table 2. Physico – chemical parameters of water sample

S.No	Stations	pH	EC/mS	DO/ppm
1	S1	7.07	0.10	03.5
2	S2	7.10	0.10	03.9
3	S3	7.5	0.10	03.6
4	S4	7.25	0.10	04.4
5	S5	7.20	0.10	03.8

pH – Concentration of Hydrogen ions
EC – Electrical conductivity
DO – Dissolved Oxygen

Table 3. RAPD – PCR products obtained with CRA 22 primers for different isolates of fresh water cyanobacteria

Phormidium lucidum SG01	*P. lucidum* SG02	*P. lucidum* SG03
750 420 305 300 295 285 280 270 260	780 600 420 305 300 290 285 270	610 410 290 240
Total 9	8	4

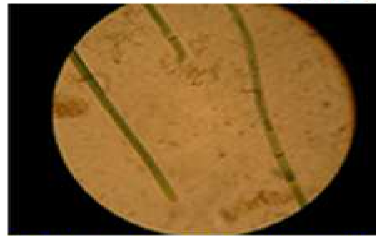


Fig.1. ***Phormidium lucidium***

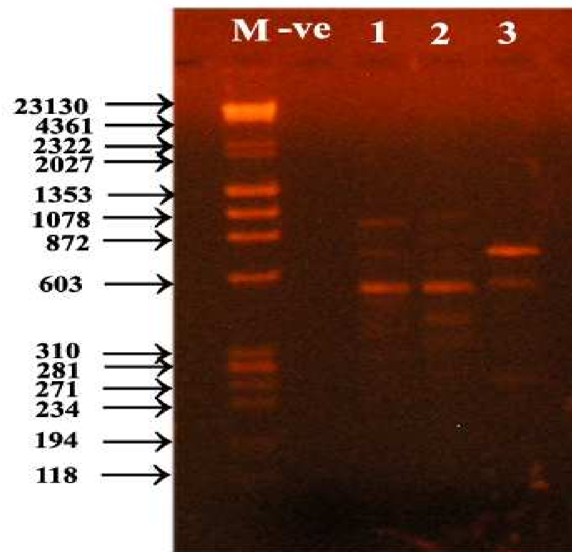


Fig.2. RAPD - PCR Banding Pattern for CRA 22

Lane M - Molecular DNA standard
Lane - ve - Negative control
Lane 1 - *Phormidium lucidium* SGO1
Lane 2 - *Phormidium lucidium* SGO2
Lane 3 - *Phormidium lucidium* SGO3

RAPD analysis

RAPD profiling has been widely accepted as valid taxonomic and phylogenetic tool for rapid and sensitive delineation of plant, animal, algae and bacterial cells [22]. Only on primer (CRA22) which exhibited reproducible polymorphism (240 – 780 bp) was selected for the detailed study. The primer CRA22 produced a unique fingerprinting for all the cyanobacterial culture (Fig. 2). [11]suggested the RAPD primers yielded 100% polymorphism among the studied strains, indicating a considerable degree of intra-specific genomic heterogeneity, with percent similarity between 13 and 82%.

The primer amplified a DNA fragment of size around 11,500bp. RAPD analysis of three Cyanobacterial isolates using CRA22 primer generated a total of 25 bands that were scored (Table 3). The band of 575 bp was recorded in three isolates. *Phormidium lucidum* SG02 produced the highest of 11,500 bp bands among the isolates. The lowest number of band 280 bp was present in *Phormidium lucidum* SG03. The banding patterns with their sizes in base pairs for the Cyanobacterial isolates tested (Fig– 3).[8], suggested the genetic relationship between *Phormidium* organisms originating from distinct geographical sites using RAPD and RFLP

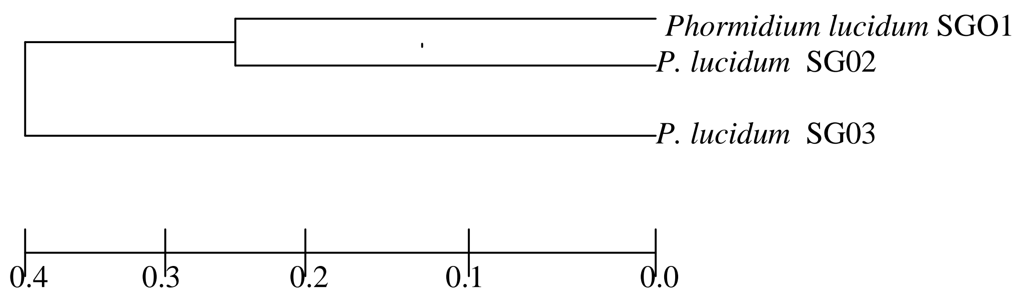
method. In the present study indicated the potential use of RAPD markers as a rapid method to detect genetic variation and the genetic relatedness of the cyanobacterial strains at the level of DNA.

Phylogenetic analysis

The data (band presence or absence) were introduced in the form of a binary matrix and a pair wise similarity [4] was constructed using the DICE coefficient (SD) [9]. SD values were obtained by the double number of shared bands between two patterns divided by the sum of all the bands in the same pattern (Value 1 indicates identical patterns for two individuals and value 0 indicates completely different pattern). The UGPMA (Unweighed pair group method with arithmetical averages) grouping of the SD values was generated using the NJ Tree program (Figure – 3).

Phenogram generated revealed two clusters A and B. The cluster A was further divided into two sub clusters A_1 and A_2. The sub cluster A consisted two strains (*Phormidium lucidum* **SG01** and *P. lucidum* **SG02),** and the cluster B having only one strain (*Phormidium lucidum* **SG03).** From the banding pattern, it was clearly shown that all the strains tested were different from each other.

Fig :3. Phenogram constructed based on RAPD banding patterns from the cyanobacterial isolates tested using CRA 22 primer



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