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## Isolation, purification and physicochemical parameters of cyanobacteria from marine environment

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### Abstract

In the current investigation analysed for diversity of cyanobacteria from east coast environment and physicochemical parameters of soil were analysed from the four different study sites. The soil physicochemical character like pH, salinity, Electrical conductivity, Organic Carbon, Organic Matter, Available Nitrogen, Phosphorus, Zinc, Copper, Iron, Manganese, Calcium, Magnesium and Potassium were experimentally performed from Kelathottam, Mallipattinam, Manora and Sethupasathiram of thanjavur district conducted. The soil has extraordinary content of nutrients were presented in keelathottam area when compared with other places. The isolation of cyanobacteria like *Anabaena azollae*, *Chroococcus limneticus*, *Dermocarpa* sp., *Gloeocapsa magma*, *Johannesbaptistia* sp., *Gloeotheca* sp., *Katagnymene* sp., *Microcoleus vaginatus*, *Myxosarcina* sp., *Nostoc muscorum*, *Oscillatoria spongelliae*, *Plectonema phormidiuodes*, *Pseudanabaena* sp., *Spirulina* sp., *Stigonema* sp., *Symploca* sp., *Synechococcus* sp., *Trichodesmium* sp and *Xenococcus* sp were recorded from four different places of Keelathottam, Mallipattinam, Monera and Sethupasathiram of Thanjavur district were analysed. Among the four places the keelathottam has maximum number of colonies and species recorded than the other places. The diversity of cyanobacteria has excellent microbial resources of our country and back bone of the marine environment.

**Keywords:** Cyanobacteria, physicochemical parameters, marine soil

### 1. Introduction

Cyanobacteria are prokaryotic, oxygen evolving, unicellular or filamentous microorganisms among which some can fix atmospheric nitrogen. They are phylogenetically related to eubacteria and algae because cyanobacteria share the characteristics of both. Green algae are eukaryotic lower plants. Both cyanobacteria and green algae show similarity in autotrophic mode of nutrition through photosynthesis. Many cyanobacteria and green algae are surrounded by a special mucilaginous covering around their cells or filaments.

Microalgae comprise of a large and diverse group of simple photoautotrophic organisms ranging from unicellular to multicellular forms. Since they have efficient access to water, carbon dioxide and other nutrients, they are generally more efficient in converting solar energy into biomass. Even if they are simple and small in structure, their build up comprises important components like carbohydrates, lipids, proteins and nucleic acids. These features bring the eyes from all the different field like biofuel, food and medicine to fall on the algal biomass.

Marine cyanobacteria have an ancient marine history which can be traced back almost three billion years ago in the fossil record during the precambrian period (Brock, 1973) <sup>[1]</sup>. They are important primary producer, and without them no animal populations including fishes could exist in natural waters (Post, 2006) <sup>[2]</sup>. They are also called as blue green algae and are widely distributed in the natural ecosystems such as land, soil, fresh water, oceans, estuarine salt lakes, salt marshes and also in hypersaline salt pans (Fogg *et al.*, 1973) <sup>[3]</sup>. They are one of the important coastal resources and constitute integral and major component of the microbiota in mangrove ecosystem along the tropical coasts (They colonize any submerged surface of sediments, roots, aerial roots, branches and trunk of mangroves (Zuberer and silver, 1978; Kathiresan and Bingham, 2001; Palaniselvam, 1998) <sup>[4, 5, 6]</sup>. However, the cyanobacterial population of parangipettai mangroves in relation to the environmental characters is explored properly. In this context the present investigation was aimed to study diversity of different four sites of cyanobacteria in marine environment.

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## 2. Materials and methods

### 2.1 Collection of water sample (Stainer *et al.*, 1971) <sup>[7]</sup>

Water sample were collected from four different sites of east coast of marine environments and were stored in chamber at 25 °C under 8 hours/16 hours dark/ light photoperiod. For morphological studies, the Cyanobacterial strain were cultivated in BG 11 medium

### 2.2 Physicochemical parameters of soil samples (APHA, 1985) <sup>[8]</sup>

Physicochemical analysis of the samples were carried out by the standard method. The rainfall data were obtained from the meteorological centre of Atherampattinam coast of Tamil Nadu, east coast of India. The atmospheric temperature and water temperature were measured by using thermometer. The electronic pH pen was used for measuring pH from sea water, and salinity by ATAGO hand refractometer. Dissolved oxygen and nutrients were estimated by Strickland and Parsons, (1972) <sup>[9]</sup>.

### 2.3 Isolation, identification and purification of Cyanobacteria

The collected sample were transferred in 100ml of BG11 medium. The flask were kept under sufficient light (1000 lux) and incubated in the flask under room temperature (22-28°C) with a PH of 8.2±1. After 15-18 days, green discoloration was seen in the culture tubes due to the growth of microalgae. The cyanobacterial sample was collected and diluted using sterile water to 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> respectively (0.1 mL) of the diluent was inoculated using pore plate method. The culture was incubated at 25±2 °C under continue illumination (3,000 lux) of light source.

Algal species were isolated from four different sites of marine environment. According to standardized algal isolation procedure (Rippka *et al.*, 1979) <sup>[10]</sup>. Identification was done using the keys given Cyanophyta by Desikachary, (1959) <sup>[11]</sup>. Pure culture of Cyanobacteria was obtained standard planting and streaking techniques (Stainer *et al.*, 1971) <sup>[7]</sup>.

## 3. Results and discussion

The highest population of algae was recorded in September, followed by July, October, June and August populations in decreasing order. The greater abundance of Chlorophyta may be related to the acid nature of the water, a reflection of the acid soil reported by (Chukwu 2007) <sup>[12]</sup>. In addition, high light intensity characteristic of the tropics favours the development of Chlorophyta. The observed algal groups (Bacillariophyta, Chlorophyta, Cyanophyta and Euglenophyta) are in agreement with the observations of (Pantastico, and Suayan 1974) <sup>[13]</sup> on Phillipines rice fields; (Fonge *et al.*, 2012) <sup>[14]</sup> in Assint rice fields, Egypt; and (Issa *et al.*, 2000) <sup>[15]</sup> as the major groups of algae in freshwater ecosystems

In the recent research analysed for soil physicochemical parameters and diversity of cyanobacteria were analysed. According to the soil physicochemical parameters like pH, salinity, Electrical conductivity, Organic Carbon, Organic Matter, Available Nitrogen, Phosphorus, Zinc, Copper, Iron, Manganese, Calcium, Magnesium, Sodium and Potassium were performed in four different soils. All the four soils keelathottam has rich nutrients can accumulated than the other three places of Mallipattinam, Manora, and Sethupasathiram of Thanjavur district. The keelathottam soil has more lagunes

and other natural invaders adapted area. so, keelathottam soil rich nutrients were observed (Table 1).

Investigated in various water sample from different regions of Vapi, Valsad, Surat, Gujarat, India were screened on algal culture plate and a total of 8 algal isolate were isolated in pure form. The pure form of the isolate were maintained in 250 ml Erlenmeyer flask containing 100 ml algal culture medium at room temperature under continuous dark and sunlight period. The morphological characteristics and its identification of cyanobacteria.

Identify the potential of protease inhibition by different genera of cyanobacteria from Central India. The investigated that a high number of cyanobacteria metabolites, including protease inhibitors have been isolated from a variety of natural samples and cultured strains. More than 80 structural archetypes of compounds belonging to more than 30 genera of all five sub sections of cyanobacteria have been defined. However, this distribution reflects the ability of stains and exploitable biomass from natural habitats. In this way, the potential to produce a protease inhibitor by theses genera often not comparable. (Raipuria *et al.*, 2016) <sup>[16]</sup>.

The morphological characteristics of the eight cyanobacterial species applied in this study. The isolate cyanobacteria was recorded from water sample of Vapi. Eight morphotypes were observed with heterocystous and non heterocystous cyanobacterial morphology.

(Mayur *et al.*, 2017) <sup>[17]</sup>.

The soil algae are an effective in the terrestrial ecosystems through its importance to the plant, by nitrogen fixation and have the ability to improve soil quality and fertility (Hussain *et al.*, 2009) <sup>[18]</sup>. A number of identified species in the current study reaches 26, they belong to 8 genera.

The domination is to the *Oscillatoria* sp. 50%, followed by *Phormidium* sp. 19.230%, followed by *Chroococcus* sp., *Spirulina* sp. 7.692%, and finally *Gloeocapsa* sp. and *Microcoleus* sp. and *Shizothrix* sp. 3.864%. Some species of Cyanobacteria recorded dominance in all areas represented by *Oscillatoria acuta*, *O. Laetevirens*, *O. tenuis*, *Phormidium anamala*, *P. pachydermiticum* (Haider *et al.*, 2017) <sup>[19]</sup>.

In the current investigation suggested that the diversity of cyanobacteria like *Anabaena azollae*, *Chroococcus* sp., *Dermocarpa* sp., *Gloeocapsa* sp., *Johannesbaptistia* sp., *Gloeothece* sp., *Katagnymene* sp., *Microcoleus vaginatus*, *Myxosarcina* sp., *Nostoc* sp., *Oscillatoria* sp., *Plectonema* sp., *Pseudanabaena* sp., *Spirulina* sp., *Stigonema* sp., *Symploca* sp., *Synechococcus* sp., *Trichodesmium* sp and *Xenococcus* sp were maximum number of colonies 86 and *stigonema* sp isolated and identified from Keelathottam Mallipattinam, Manora and Sethupasathiram area. Among the four places, keelathottam has maximum diversity of cyanobacteria were determined than the other places. However, the keelathottam soil nutrient content has excellent with diversity of cyanobacteria also high number of colonies were analysed. So, nutrient content of the soil has recognized factors for the population of microbiota (Table 2).

The values of the Shannon Index recorded in the present study are higher than those recorded by (Prasanna 2007 <sup>[20]</sup> and Ahad *et al.*, 2015) <sup>[21]</sup> in India. The results of the statistical analysis showed significant differences between areas because of changes in soil cyanobacteria species usually was a quantitatively, this is due to fluctuation in water availability while the composition of species is constant (. Metting 1981) <sup>[22]</sup>, (Table 3).

**Table 1:** Physico-chemical properties of soil sample of south east coast of India

S. No	Name of the parameter	Different places			
		Kelathottam	Mallipattinam	Manora	Sethupasathiram
1.	pH	8.0	8.0	7.9	8.0
2.	Electrical conductivity (dsm <sup>-1</sup> )	0.26	0.28	0.29	0.26
3.	Organic Carbon (%)	0.16	0.28	0.19	0.23
4.	Organic Matter (%)	0.32	0.26	0.24	0.23
5.	Available Nitrogen (mg/kg)	73.8	62.7	63.4	63.5
6.	Available Phosphorus (mg/kg)	4.0	3.5	3.4	3.3
7.	Available Potassium (mg/kg)	110	112	105	102
8.	Available Zinc (ppm)	1.02	1.32	1.15	1.16
9.	Available Copper (ppm)	0.52	0.57	0.54	0.56
10.	Available Iron (ppm)	4.62	3.95	3.87	3.51
11.	Available Manganese (ppm)	1.31	1.25	1.16	1.17
12.	Calcium (C. Mole Proton <sup>+</sup> /kg)	1.3	0.2	0.1	0.6
13.	Magnesium(C. Mole Proton <sup>+</sup> /kg)	6.5	5.2	5.1	4.3
14.	Sodium (C. Mole Proton <sup>+</sup> /kg)	1.2	1.6	1.4	1.3
15.	Potassium(C. Mole Proton <sup>+</sup> /kg)	0.2	0.8	0.7	0.4

**Table 2:** Isolation and identification of cyanobacteria from east coast of marine environments

S. No	Name of the cyanobacteria	Kelathottam	Mallipattinam	Manora	Sethupasathiram
1.	<i>Anabaena azollae</i>	2	5	-	-
2.	<i>Chroococcus limneticus</i>	7	-	7	9
3.	<i>Dermocarpa</i> sp	-	6	-	-
4.	<i>Gloeocapsa magma</i>	-	3	-	-
5.	<i>Johannesbaptistia</i> sp	4	-	4	3
6.	<i>Gloeotheca</i> sp	-	3	-	-
7.	<i>Katagnymene</i> sp	3	-	-	-
8.	<i>Microcoleus vaginatus</i>	2	-	2	3
9.	<i>Myxosarcina</i> sp	-	2	5	-
10.	<i>Nostoc muscorum</i>	8	-	7	9
11.	<i>Oscillatoria spongelliae</i>	10	-	-	-
12.	<i>Plectonema phormidiuodes</i>	11	4	-	6
13.	<i>Pseudanabaena</i> sp	12	7	-	3
14.	<i>Spirulina</i> sp	7	8	6	4
15.	<i>Stigonema</i> sp	3	6	8	4
16.	<i>Symploca</i> sp	4	5	-	2
17.	<i>Synechococcus</i> sp	-	-	7	1
18.	<i>Trichodesmium</i> sp	6	-	6	3
19.	<i>Xenococcus</i> sp	7	4	5	4
Total no of colony		86	53	57	51

**Table 3:** Diversity indices of cyanobacteria from different area

Area	No. of Species	No. of individuals	Margalef index (d)	Menhinick's species richness (SR)	Pielou Evenness (J')	McIntosh Evenness (McE)	Shannon's Diversity (H')	Margalef Diversity (Dmg)
Kelathottam	86	14	15.428	12.286	0.057	0.068	0.082	55.227
Mallipattinam	53	12	9.438	7.571	0.063	0.090	0.060	61.960
Manora	57	11	10.164	8.143	0.062	0.086	0.050	60.845
Sethupasathiram	51	12	9.075	7.286	0.064	0.092	0.060	62.566
Total	247	49	44.107	35.286	0.246	0.336	0.252	240.599

#### 4. Conclusion

It can be concluded that the degradation of many proterozoic oil deposits are attributed to the activity of cyanobacteria. They are also important providers of nitrogen fertilizer in the cultivation of crops because they are photosynthetic and aquatic cyanobacteria indispensable for the environment. Due to their ability to produce oxygen, cyanobacteria played a pivotal role in changing the composition of atmospheric nitrogen in plants.

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