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RESEARCH ARTICLE

Evaluation of Cyanobacterial Distributions in Estuary Region of Southeastern India and its Phycoremediation Studies in Industrial Effluent

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Abstract

Cyanobacteria are photo-autotrophs, potential rich organisms gaining interest in all fields. They are almost cosmopolitan in moisture rich environment, holding different adaptations based on their locations and encompass wide potentials which are boon to that ecosystem. Marine cyanobacteria contribute its role in various ways such as energy fuel, food, medicine and also in bioremediation process. The study focused on seasonal distribution of cyanobacteria in Arumuganeri estuary region, Tuticorin district, Tamil Nadu, India. Phycoremediation studies were performed against distillery effluent using marine cyanobacterial isolates which are obtained from the enrichment. Morphological characterization was done. A total of 36 cyanobacterial species belongs to five families were observed in those sites. Among them, the cyanobacterial species such as *Spirulina subsalsa, Oscillatoria willei* and *Lyngbya aestuarii* were plays vital role in degradation.

INTRODUCTION

About 70.68% our planet earth is occupied by oceans. India is one of the sub continent possess wide region of an exclusive economic zone (EEZ) extending to 2.02 million km^2. Such marine environment exhibits various types of settings such as shallow sea, benthic regions, estuaries, lagoons and saltpan (Das and Desai 1988; Nedumaran, 2009).

Estuaries are the regions were fresh water finally mixes with seas and oceans, exhibit shifting salinity from oligohaline to mesohaline (Schuchardt et al. 1999). Thamiraparani is one of the perennial rivers in Tamilnadu come from Western Ghats and run towards southeastern coastal region of Tamilnadu, India. Tides in the estuary region are of semidiurnal type about -0.6m near the mouth region, showing substantial range and time.

Cyanobacteria are Gram-negative prokaryotes but possess peptidoglycan bound cell wall like eubacteria. They exists in aggregated mat consists of different morphologies such as unicellular and filamentous forms (Castenholz, 2001). A numerous studies were gone through the distribution of cyanobacteria and potentials according to various environments (Bergman et al. 1997; Warwick 2000). Cyanobacterial species are capable of growing on both soil and water conditions and plays an imperative role in that ecosystem (Ray, 2006). So far many potential cyanobacterial species were identified from both freshwater and marine ecosystems (Muthukumar et al. 2007).

Marine cyanobacteria are ability to tolerate high salt concentrations. Inspite of its hypersaline growing nature, saltpans Marine cyanobacteria has biofertilizer ability and reclamation of saline or alkaline rich soil (Sugumar et al. 2011). Recent studies show that cyanobacteria in estuary region gained interest and being explored widely due its significant potential bio-active compounds (M. C. Murrell and E. M. Lores, 2004; Joseph, 2005).

Cyanobacterial species shows collective dominance even in in turbid water and low light intensity to maintain buoyancy and the capacity to grow exponentially in wet period in which nitrogenous nutrients were high (Chellappa and Costa 2003; Lacap et al. 2005; Nedumaran and Prabu 2009). Due to the high levels of combined nitrogen in the sea, the heterocystous forms were rarely seen in the east coast of India (Thajuddin and Subramanian, 1990; Nagasathya and Thajuddin 2008). Selvakumar and Sundararaman (2007) observed major cyanobacterial species are belonging to either Chroococcaceae or Oscillatoriaceae families in estuarine water.

The cyanobacteria possess remarkable adapting ability to salinity and other environmental factors (Desikachary, 1959; Carr and Whitton, 1982). Hypersaline environment namely salterns shows wide range of cyanobacterial species and the maximum diversity of the cyanobacterial flora in the Gulf of Mannar region correlated well with the higher salinity, pH and nutrient content of the water (Subramanian and Thajuddin, 1995; Thajuddin and Nagasathya, 2008; Su et al. 2014).

Cyanobacteria can enhance soil fertility with aid of nitrogen fixation and phycoremediation (Karthikeyan et al. 2007; Vijayakumar et al. 2011). They also used in soil reclamation process by reducing the salinity stress induced in crops while treated with brackish water containing 20% (v/v) sea water (Cohen. 2002; Rodríguez et al. 2006; El-Baky et al. 2008; Jing et al. 2009). The cyanobacteria also having vital role in bioremediation roles such as CO_2 consumption and effluent treatment (Dubey et al. 2011; Hegazi et al. 2010). The present studies focused on the ecological distribution of marine cyanobacteria in one estuary sites and their effluent treatment studies.

Materials and Methods

Sampling sites

The visible cyanobacterial mat samples were collected at six different sites of estuary region near Arumuganeri ($8^0$32’05.65” N, $78^0$06’36.60” E) arised by mixing of *perennial river Thamiraparani* comes under southeastern coastal region of Tamilnadu, India. From the six sites, samples were bi annually collected for three consecutive years since August 2011 to August 2014. Such samples were maintained and analysed its cyanobacterial distribution were previously mentioned in previous studies (Sugumar et al. 2011; Ramanathan et al. 2013).

Cultivation and Purification:

Cyanobacterial mats were inoculated and maintained in ASN III, MN II and BG 11 marine broth (Rippka et al. 1979). They were incubated with an illumination of white fluorescent lamps of 2500 lux at 25^{o}C for 20 days. The cultures were made pure by repeated and frequent subculturing in BG 11 medium, plated on solid cyanophycean agar medium with same illumination source and temperature for 18hrs a day (Vijayakumar et al. 2007).

Distribution and identification:

Cyanobacterial specimens were observed by microscopic observations and identified by using the publications of Desikachary (Desikachary, 1959). Enumeration and seasonal distribution of marine cyanobacteria estuary site were observed. Various types of diversity indices like Simpson’s index, Margalef index, McIntosh index, Pielou evenness & McIntosh Evenness of six sites were calculated (Turkmen and Kazanci, 2010).

Effluent treatment:

The cynobacterial mat were placed in a tray containing industrial effluent and kept for incubation for one week. After one month enrichment, the cultures were plated on solid cyanophycean agar medium. Inoculated plates were incubated and then identified cyanobacterial species grown in it. The obtained species were inoculated individually into 100-ml culture medium containing 5% effluent kept for 1 week and observed its growth.

Specific Growth rate based on Biomass:

The specific growth rate (μd^{-1}) was calculated as follows:

$$? = \frac{In\left(\frac{Wf}{Wo}\right)}{\Delta t}$$

Where W_f and W_o were the final and initial biomass concentration respectively. Δt was the cultivation time in day (Ono and Cuello 2007).

Biomass productivity of marine cyanobacteria:

Biomass productivity was calculated by subtracting the initial dry weight from the final dry weight divided by the number of days the cultures were grown.

$$P = \frac{\text{Initial dryweight} - \text{Final dryweight}}{\text{Incubation days}}$$

Results and Discussion:

About 36 species of cyanobacteria from 14 genera belonging to 5 families were observed of which, 13 were unicellular colonial forms and 23 nonheterocystous filamentous forms. Interestingly, maximum number of species about 23 species of 12 genera was recorded from summer season (Table 1).

Major marine Cyanobacteria genera from Arumuganeri estuary are: *Chroococcus, Microcystis, Synechococcus, Spirulina, Oscillatoria, Phormidium, Lyngbya, Microcoleus* and *Calothrix.* Among the cyanobacterial species, *Chroococcus minutes*, *Oscillatoria chlorina* , *Phormidium fragile* and *Lyngbya aestuarii* were observed in all the period of collections which are considered as versatile species (Table 1). The various types of diversity indices were calculated and tabulated (Table 2). Interestingly, cyanobacterial species were evenly distributed in both estuaries site.

After one week treatment, three cyanobacterial species were *Spirulina subsalsa, Oscillatoria willei* and *Lyngbya aestuarii* observed in the effluent samples. While treating the effluent with individual organisms, they all exhibited highly efficient degradation or removal of effluent concentrations. Biodegradation capabilities were observed as individual or mixed cultures.

The growth of cyanobacteria was measured at equal time intervals using optical density. *Spirulina subsalsa*, *Oscillatoria willei* and *Lyngbya aestuarii* were tested under effluent. *Spirulina subsalsa* obtained faster growth rate than the *Oscillatoria willei* and *Lyngbya aestuarii* with the effluent. *Oscillatoria willei* and *Lyngbya aestuarii* growth rate was slower effluent treatment.

The specific growth rate, µ, is a measure of how quickly a microbial population is growing. Table 4 shows that the specific growth rate based on biomass of marine cyanobacteria with effluent. The effluent treated *Spirulina subsalsa*, were reached the specific growth rate of about, 0.069 µ d^{-1} based on biomass. The specific growth rate of *Oscillatoria willei* and *Lyngbya aestuarii* indicates that their growth rate was slower with the effluent treatment.

Biomass productivity rate by the three cyanobacterial species which cultivated effluent was calculated and shown in table 5. Among the 3 species, produced higher biomass *Spirulina subsalsa* (0.43 $mg.m1^{-1}.\ d^{-1}$) than *Oscillatoria willei* (0.4 $mg.m1^{-1}.\ d^{-1}$), *Lyngbya aestuarii* produced (0.35$mg.d^{-1}.m1^{-1}$) from the effluent treatment.

Our previous study ensures that cyanobacteria species can enhance the plant growth by both directly and indirectly. Likewise it also made improvement of rice seedling of wheat crops while treated with brackish water containing 20% (v/v) sea water.

The biomass growth confirms those *Spirulina subsalsa* which are pollution tolerances and also decolourization in medium validated that nutrients in the effluent are utilized by the mictobes which are said to be phycoremediation. Salinity and pH was found to be positively correlated with cyanobacterial numbers. Thus it makes marine cyanobacterial species are facultative halophiles. While the salinity increases it shows abundance growth than the monsoon seasons. In otherwords, estuary region performs a enrichment of salinity adaptation of marine cyanobacteria.

The present studies showed that diversity of cyanobacterial species in a particular region and its all diversity indices were almost similar and highly resemble to marine cyanobacteria. Apart from it biofertilzation ability its also acts as phycoremediation in various environment at very low cost. Further studies in these marine cyanobacteria obtained from undisturbed areas are needed to find out pollution degrading pattern, to increase its rate of degradation, its phylogenetic relationship with each other and explore its potential.

Table1. Distribution of Cyanobacterial species in Arumuganeri estuary sites.

Name of the organism	**Collection period**					
	2012		**2013**		**2014**	
	SS	**RS**	**SS**	**RS**	**SS**	**RS**
Order: Chroococcales Wettstein **Family: Chroococcaceae** Nageli						
Aphanothece microscopica Nag	+	+	+	+	+	++
Aphanocapsa littoralis Hansgirg	+	+	+	+	+	+
Aphanocapsa marina	-	-	-	-	-	-
Chroococcus minutus (Kutz.) Nag	++	++	+	++	++	++
*Chroococcus turgidus (*Kutz.*)* Nag	++	-	+	+	++	+

Microcystis litoralis (Hansg.) Forti	-	-	-	+	-	+
Microcystis aeruginosus Kutz	++	-	-	-	++	-
Synechocystis salina wislouch	++	+	+	-	++	+
Synechocystis pevalekii Ercegovic.	-	-	-	-	-	-
Synechococcus aeruginosus Nag	-	-	+	+	-	+
Synechococcus elongatus Nag	+	+	+	-	+	++
Order: Pleurocapsales Geitler **Family: Pleurocapsaceae** Geitler						
Myxosarcina burmensis skuja	-	+	++	-	-	+
Myxosarcina spectabilis Geitler	-	-	-	-	-	+
Order: Nostocales Geitler **Family: Oscillatoriaceae** Kirchner						
Lyngbya aestuarii Liebm. Ex Gomont	++	+	++	++	++	++
Lyngbya ceylanica v. *constricta* Fremy	-	-	+	-	+	-
Lyngbya sps	-	-	-	+	-	-
Lyngbya lutea (Ag.) Gomont	+	+	-	-	+	-
Lyngbya tayloriae Forti	+	+	-	-	+	-
Oscillatoria brevis (Kutz.) Gomont	-	-	-	-	-	+
Oscillatoria chlorina Kutz. ex Gomont	++	+	++	-	++	++
Oscillatoria limosa Ag. Ex Gomont	-	-	+	+	-	-
Oscillatoria lemmermannii Wolosz	+	+	-	-	+	-
Oscillatoria subbrevis Schmidle	++	+	+	+	++	++
Oscillatoria salina Biswas	++	-	+	+	++	+
Oscillatoria willei Gardner em. Drouet	++	-	+	-	++	-
Oscillatoria tenuis Ag. ex Gomont	-	-	-	-	-	+
Microcoleus chthonoplastes Thuret ex Gomont	++	-	-	-	++	+
Spirulina major Kutz. Ex Gomont	++	-	++	-	++	+
Spirulina laxissima forma *major*	+	+	+	+	+	+
Spirulina subsalsa Oerst. Ex Gomont	++	-	++	+	++	++
Spirulina labyrinthiformis (Menegh.) Gomont	+	+	+	-	+	+
Phormidium fragile (Meneghini) Gomont	++	+	++	+	++	++
Phormidium corium (Ag.) Gomont	+	+	+	-	+	+
Phormidium tenue (Menegh.) Gomont	+	-	+	-	+	+
Phormidium valderianum (Delp.) Gomont	-	-	-	+	-	-
Family: Rivulariaceae Rabenhorst						
Calothrix geitonos Skuja	-	-	-	+	-	+
SS-Summer season, RS-Rainy season						

Table 2. Diversity indices of cyanobacteria in Arumuganeri estuary site.

Diversity Indices	Saltpan Site		
	2012	2013	2014

		SS	RS	SS	SS	RS	SS
Richness	Margalef index (d)	9.6	8.1	9.2	7.8	10.7	8.7
	McIntosh Index (Mc)	0.89	0.84	0.94	0.83	0.98	0.79
Evenness	Pielou Evenness (J')	0.93	0.9	0.99	0.96	0.93	0.99
	McIntosh Evenness (McE)	0.96	0.85	0.98	0.94	0.95	1.0
Diversity	Simpson's Diversity	0.96	0.92	0.94	0.91	0.97	0.95
	Shannon's Diversity (H')	3.4	2.8	2.9	2.6	3.5	2.6

SS-Summer season, RS-Rainy season

Table 3: Physio-chemical parameters of distillery spent wash effluent

Parameters	**Distillery effluent**
Colour	Dark brown – Brown
Odour	Unpleasent
pH	4.6± 0.2
EC (µmho/cm)	16452.4 ± 8.2
Total Dissolved solids	38205.2± 4.8
Total suspended solids	42150.0 + 0.2
Biological Oxygen Demand	32300.8 ± 12.2
Chemical oxygen Demand	57164.6 ± 10

Table 4: Specific growth rate (Based on biomass of marine cyanobacteria) in effluent treatment

Name of the marine cyanobacteria	**Specific growth rate based on biomass ($\mu_{biomass}$ d^{-1})**
Lyngbya aestuarii	0.057 ± 0.032
Spirulina subsalsa	0.069 ± 0.052
Oscillatoria willei	0.054 ± 0.012

Results are means ± S.E. of 3 replicates

Table 5: Biomass productivity of 5% effluent treated marine cyanobacteria

Name of the marine cyanobacteria	**Initial dry biomass (mg. ml^{-1})**	**Final dry biomass (mg.ml^{-1})**	**Biomass productivity (mg.ml^{-1} d^{-1})**
Lyngbya aestuarii	09.92 ± 0.5	12.5 ± 0.94	0.35 ± 0.032
Spirulina subsalsa	7.16 ± 1.87	10.1 ± 2.04	0.43 ± 0.14
Oscillatoria willei	4.84 ± 0.82	7.7 ± 1.07	0.4 ± 0.17

Results are means ± S.E. of 3 replicates

Fig. 1 Species richness diversity index of Reservoir region

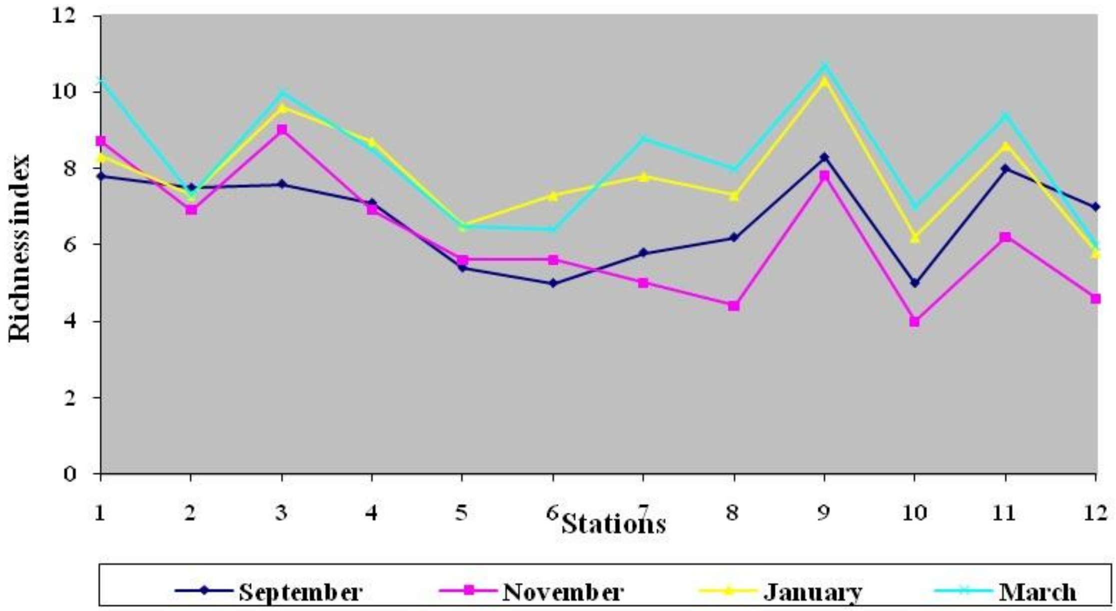
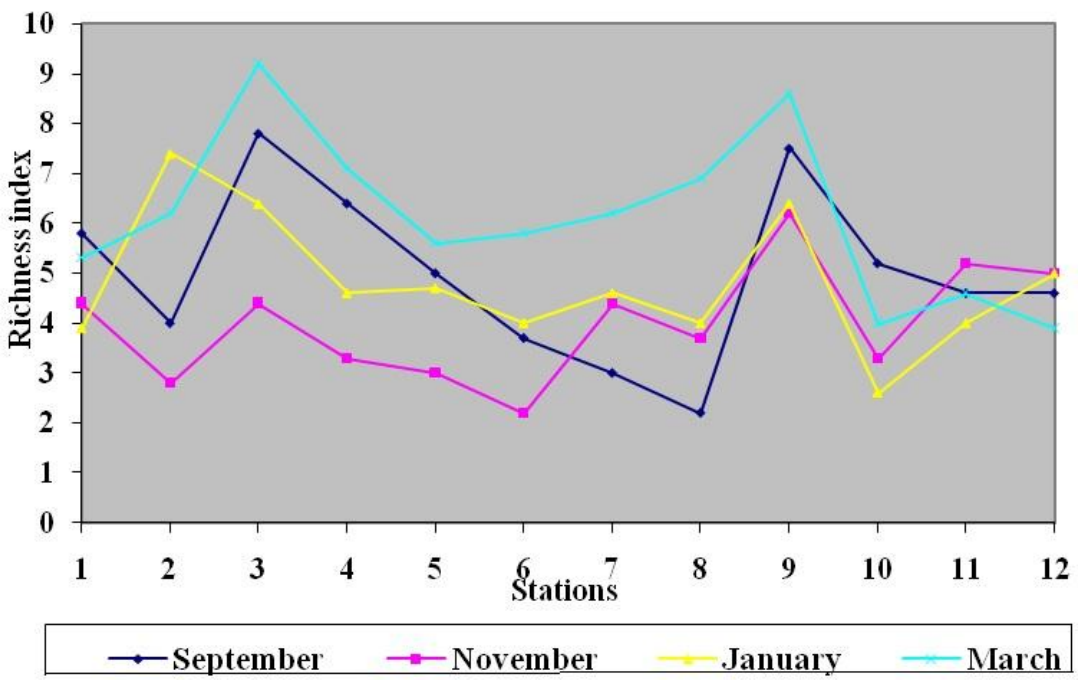


Fig. 2 Species richness diversity index of condenser region



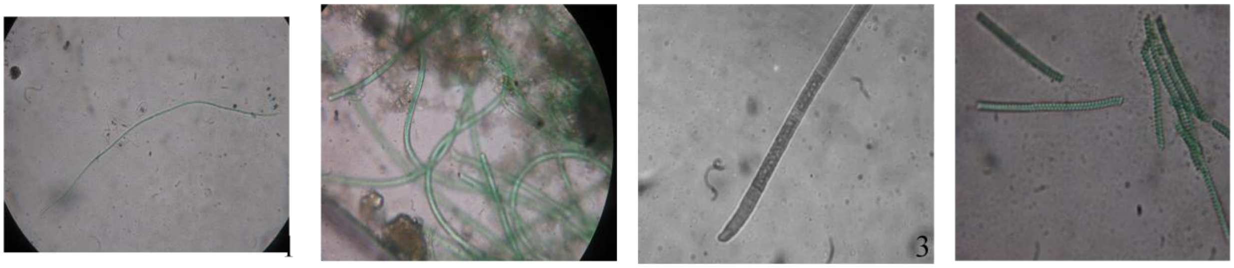


Fig. 3 Microscopic view of versatile cyanobacteria from Arumuganeri estuary site of southeast coast of Tamilnadu: 1.*Lyngbya* sps, 2. *Lyngbya aestuarii* Liebm ex Gomont, 3.*Oscillatoria willei* Gardner em. Drouet, and 4. *Spirulina subsalsa* Oerst. ex Gomont.

Conclusion:

Extreme environment enriches adaptations and biopotentials in microbes for its survival in that environment. Marine cyanobacterial occurance and distribution is directly proportional to the salinity of the medium. During monsoon season, cyanobacterial diversity is high in estuary region of south eastern coast of Tamilnadu. Halophilic cyanobacteria plays vital role in bioremediation processes. *Spirulina subsalsa*,, *Oscillatoria willei* and *Lyngbya* sps which commonly seen in all seasons in the estuary region. Among the three versatile species, *Spirulina subsalsa*, showed high biomass growth in various effluent treatment such as distillery and refinery effluent.

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