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OCCURRENCE OF NITROGEN-FIXING CYANOBACTERIA IN LOCAL RICE FIELDS OF ORISSA, INDIA

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ABSTRACT

Cyanobacterial diversity occurring in some local rice fields of Orissa, India has been studied in two different seasons and influence of pH, organic carbon (%) and conductivity were correlated on their population. At different locations and seasons the pH of the soil varies from 6.30±0.20 to 6.66±0.305. Conductivity and organic carbon (%) varies from 0.6±0.10 to 0.76±0.152 and 0.56±0.045 to 0.70±0.025 respectively. Altogether 58 taxa belonging to 20 genera were obtained and characterized. Out of these 19 forms were heterocystous and 39 were non-heterocystous. Highest abundance of cyanobacteria was found in order Nostocales which was represented by 15 species. Among the species relative abundance of *Oscillatoria chalybea* (9.90%) was found to be highest followed by *Phormidium purpurascens* (8.49%), *Cylindrospermum muscicola* and *Oscillatoria chlorina* (8.01%). Highest Shannon's diversity index was recorded in sampling site 1, followed by site 2. Comparatively the diversity index was more during winter than in summer in all the study sites. Highest similarity index (0.174) was found in summer-summer isolates of site 1 and 3. Among the environmental variables (i.e., soil pH, organic carbon (%), conductivity) highly positive correlation was observed between cyanobacterial population and soil pH ($r \geq 9$) in all the three sampling sites. The study indicates ubiquitous distribution of cyanobacteria in rice fields and could be exploited for biofertilizer in agriculture.

Key words: Cyanobacteria, rice fields, abundance, diversity index, environmental variables.

INTRODUCTION

Cyanobacteria are extraordinarily diverse group of Gram-negative, oxygenic photosynthetic prokaryotes that are distributed in all possible biotopes of the world. Due to their occurrence in diverse habitats, these organisms are excellent materials for investigation by ecologists, physiologists, biochemists, microbiologists and biotechnologists. Nitrogen fixing cyanobacteria are unique as they are able to assimilate both carbon and nitrogen. The Key enzyme nitrogenase involved closely to fix atmospheric nitrogen (Stewart *et al.* 1987). Algalization of soil with living di-nitrogen fixing cyanobacteria has become a common practice in tropical countries for many years (Venkatraman *et al.* 1974). Recently soil algalization has received an increasing attention in the temperate countries also because of its implications in reducing environmental pollution, removing soil compaction and turf treatment (Roger and Kulasooriya 1981).

Various workers have studied the cyanobacterial flora of rice fields of our country (Ahmed *et al*. 1999, Tiwari *et al*. 2000, Nayak *et* *al*. 2001, Kaushik and Prasanna 2002, Mishra and Pabbi 2004, Choudhury and Kennedy 2005, Rai 2006, Nayak and Prasanna 2007, Digambar Rao *et al*. 2008) and few attempts have also been carried to explore their diversity in the state of Orissa (Mohanty and Padhi 1984, Padhy *et al*. 1992, Sahu *et al*. 1996, Bhakta *et al*. 2006, Dey and Bastia 2008). Nevertheless, studies on cyanobacteria from the rice fields of this part of the state still remain largely unexplored. Therefore, the objectives of the present investigation were (i) Collection and identification of cyanobacteria from local rice fields, (ii) Study of cyanobacterial diversity using diversity indices in different seasons and locations, (iii) Study of physico-chemical properties of rice field soils and correlating to cyanobacterial population.

MATERIALS AND METHODS

Study area: The study was conducted in Mayurbhanj district of Orissa situated between 21°16'–23°34' North latitude and 85°40'–87°91' East longitude. Meteorological data of the study sites were collected from district agriculture and meteorology office.

Collection, identification, maintenance and preservation of samples: Samples were collected in two different seasons, i.e., summer (Rabi crop) and winter (Kharif crop) from the three different sites. The samplings were done randomly from both soil and water of the paddy fields. Temporary slides were prepared for each sample for identification. The strains were identified based on their morphological features and cell structure following the monograph of Desikachary (1959) and Anand (1989). The collected samples were maintained by culturing in freshly prepared BG-11±N medium (Rippka *et al*. 1979) and incubated at 28±2°C with illumination at 25-30 μmol photon $m^{-2}s^{-1}$ white continuous light and aeration. A part of each collected cyanobacterial samples were preserved in 4% formaldehyde solution and deposited in the Department of Botany, North Orissa University for future references.

Physico-chemical properties: The pH and conductivity of the soil samples were determined by using digital pH meter and conductivity meter, respectively. The organic carbon (%), available soil phosphorus and potash were determined by colorimetric method, Bray's I method and Ammonium acetate extraction method, respectively.

Statistical analysis: Correlation coefficient (r) between cyanobacterial population and pH, conductivity and organic carbon (%) of different sampling sites at different seasons were analyzed by calculating the regression equation and simple linear correlation coefficient.

DATA ANALYSIS

Relative abundance: The relative abundance of a particular cyanobacteria type was calculated by employing the following formula:

$$\text{Relative abundance} = \frac{Y}{X} \times 100$$

Where,

X = total number of samples collected

Y = number of samples from which a particular cyanobacteria type was isolated.

Diversity Index: The Diversity Index (Shannon-Wiener) was been studied following the formula:

$$Hs = -\sum_{i=1}^{S} (Pi)(1n\, Pi)$$

Where,

H_s - diversity in a sample of S species or kinds

S - the number of species in the sample

Pi - relative abundance of i^{th} species or kinds measures, = n_i/N

N - total number of individuals of all kinds

n_i - number of individuals of i^{th} species

ln - log to base 2

Similarity coefficient: Similarity coefficient of cyanobacteria in different sites and seasons were studied following the formula:

$$\frac{C}{A+B+C}$$

Where,

A and B represent number of species present at any two different study sites

C = Common species between any two study sites.

RESULTS

The physico-chemical properties of the rice fields soil like pH, conductivity, organic carbon (%), phosphorus and potash varies from different sites and seasons (Table 1). At different locations and seasons the pH of the soil varies from 6.30±0.20 to 6.66±0.305. Conductivity and organic carbon (%) varies from 0.6±0.10 to 0.76±0.152 and 0.56±0.045 to 0.70±0.025, respectively. Similarly, phosphorus and potash contains varies from 6.40±1.058 to 9.47±0.97 and 225±13.8 to 335±23.6, respectively.

During the present investigation 212 samples were collected from three different study sites and a total of 58 taxa belonging to 20 genera were characterized. Out of these 19 forms were heterocystous and 39 were non-heterocystous belonging to 2 orders namely Chroococcales and Nostocales of Cyanophyta. *Oscillatoria chalybea* (9.90%) was the most abundant cyanobacteria followed by *Oscillatoria subbrevis* (8.96%), *Phormidium purpurascens* (8.49%), *Cylindrospermum muscicola* (8.01%), *Oscillatoria chlorina* (8.01%), *Anabaena constricta* (5.66%), *Oscillatoria princeps* (5.18%) and *Oscillatoria animalis* (4.71%) (Table 2). Among the locations, site 1 was recorded with highest numbers of cyanobacterial strains in both the season, followed by site 3 and 2. The number of isolates was more in winter than in summer in all the locations.

Diversity index of cyanobacterial populations occurring in rice fields at different seasons and locations were calculated by Shannon-Wiener method (Table 3). Among the locations, site 1 was found to be rich in cyanobacterial population in both the seasons. During the winter season, number of isolates were more than the summer season with diversity index of 3.357 and 3.184, respectively. Similar observations were made for different locations at site 2 and 3, with diversity index of 3.295 and 3.257 during the winter and 2.610 and 2.878 during the summer, respectively. Similarity Co-efficient between different seasons and location were calculated following Jaccard's Similarity Co-efficient method (Table 4). Highest similarity index (0.174) was found in summer-summer isolates of location site 1 and 3. Lowest similarity index (0.044) was observed in summer-summer isolates of location site 2 and 3. Higher values of Similarity Co-efficient indicate the occurrence of common species during this particular season. Correlation and regression analysis were carried out between the environmental variables (i.e., soil pH, organic carbon (%), conductivity) and cyanobacterial population in different study sites. Correlation has been observed between cyanobacterial population and soil pH ($r \geq 9$) in all the three sampling sites (Fig. 1a). However, the cyanobacterial population did not show positive correlation to organic carbon (%) and conductivity in the study sites (Figs. 1b and 1c).

Table 1. Physico-chemical properties of rice field soils at different locations.

Location	Season	pH	Conductivity	Organic carbon %	Available Phosphorus	Available potash
Site-1	Summer	6.30±0.20	0.6±0.10	0.56±0.045	7.86±1.29	282±25.65
	Winter	6.53±0.252	0.7±0.10	0.70±0.025	8.43±1.16	320±29.90
Site-2	Summer	6.36±0.208	0.63±0.152	0.63±0.065	8.37±0.503	225±13.8
	Winter	6.66±0.256	0.7±0.20	0.69±0.027	9.47±0.97	335±23.6
Site-3	Summer	6.5±0.20	0.76±0.152	0.56±0.095	9.23±1.106	209±22.7
	Winter	6.66±0.305	0.66±0.152	0.63±0.611	6.40±1.058	328±41.94

All the values are mean ± S.D, n = 3. Site-1 = Baripada Block, Site-2 = Suliapada Block, Site-3 = Muruda Block. Available phosphorus and potash was calculated in Kg/hectare

Table 2. Seasonal variations and abundance (%) of cyanobacterial flora of local rice fields in the three study sites.

SN	Cyanobacteria type	S-1		S-2		S-3		Total	Abundance
		S	W	S	W	S	W		%
1	*Aphanocapsa biformis*	-	1	-	-	-	-	1	0.47
2	*Aphanocapsa crassa*	-	1	-	2	1	-	4	1.88
3	*Aphanocapsa banaresensis*	-	-	-	-	1	2	3	1.41
4	*Aphanocapsa elachista*	-	-	-	-	-	1	1	0.47
5	*Aphanothece microscopic*	-	1	-	2	-	-	3	1.41
6	*Chroococcus cohaerens*	1	-	2	-	-	1	4	1.88
7	*Microcystis aeruginosa*	-	1	1	-	-	-	2	0.94
8	*Microcystis bengalensis*	1	2	1	-	-	-	4	1.88
9	*Microcystis* sp.	-	-	-	1	-	3	4	1.88
10	*Dactylococcopsis* sp.	-	-	-	-	-	1	1	0.47
11	*Microchaete aequalis*	1	2	-	-	1	1	5	2.35
12	*Anabaena constricta*	2	3	-	4	-	3	12	5.66
13	*Anabaena variabilis*	1	2	-	3	1	1	8	3.77
14	*Anabaena circinalis*	1	2	-	2	-	4	9	4.24
15	*Anabaena spidoides*	-	-	-	1	2	3	6	2.83
16	*Anabaena naviculoides*	1	3	-	-	1	-	4	1.88
17	*Anabaena fuellebornii*	1	1	-	2	1	-	5	2.35
18	*Nostoc spongiaeforme*	-	3	-	2	-	1	6	2.83
19	*Nostoc piscinale*	1	2	-	3	1	1	8	3.77
20	*Nostoc calcicola*	-	4	-	-	1	-	5	2.35
21	*Nostoc hatei*	-	-	--	1	-	-	1	0.47
22	*Cylindrospermum stagnale*	1	1	1	2	-	-	5	2.35
23	*Cylindrospermum muscicola*	2	7	1	4	-	3	17	8.01
24	*Cylindrospermum indicum*	2	3	-	1	1	2	9	4.24
25	*Cylindrospermum licheniforme*	1	-	1	-	-	-	2	0.94
26	*Cylindrospermum sphaerica*	-	2	1	-	-	2	5	2.35
27	*Cylindrospermum* sp.	-	-	-	1	-	-	1	0.47
28	*Aulosira fertilissima*	1	2	1	1	-	-	5	2.35
29	*Spirulina gigantea*	-	1	1	-	-	2	4	1.88
30	*Oscillatoria chalybea*	2	7	-	5	1	6	21	9.90
31	*Oscillatoria subbrevis*	3	6	3	2	-	5	19	8.96
32	*Oscillatoria princeps*	1	4	-	2	1	3	11	5.18
33	*Oscillatoria margaretifera*	1	1	-	2	-	3	7	3.30

34	*Oscillatoria chlorine*	2	8	-	3	1	3	17	8.01
35	*Oscillatoria animalis*	1	3	1	-	3	2	10	4.71
36	*Oscillatoria acuminate*	-	4	2	1	-	-	7	3.30
37	*Oscillatoria acuta*	2	3	1	-	1	-	7	3.30
38	*Oscillatoria curviceps*	-	1	-	-	-	2	3	1.41
39	*Oscillatoria willei*	-	1	1	-	-	-	2	0.94
40	*Oscillatoria laetevirens*	-	-	-	2	1	-	3	1.41
41	*Oscillatoria homogenea*	-	1	-	1	-	2	4	1.88
42	*Oscillatoria trichoides*	-	2	-	-	-	1	3	1.41
44	*Oscillatoria* sp.	-	2	-	-	-	1	3	1.41
45	*Pseudanabaena schmidlei*	-	-	-	1	-	-	1	0.47
46	*Phormidium purpurascens*	2	5	1	4	-	6	18	8.49
47	*Phormidium stagnina*	1	2	-	1	-	3	7	3.30
48	*Phormidium rotheanum*	-	-	-	3	-	1	4	1.88
49	*Phormidium corium*	1	1	-	-	1	2	5	2.35
50	*Phormidium* sp.	-	2	1	3	-	-	6	2.83
51	*Lyngbya ceylanica*	1	2	-	2	-	-	6	2.83
52	*Lyngbya sordid*	1	1	-	-	-	-	2	0.94
53	*Symploca hydnoides*	-	-	-	1	-	-	1	0.47
54	*Schizothrix telephoroides*	-	-	-	2	-	-	2	0.94
55	*Hydrocoleum heterotrichum*	-	1	-	3	-	-	4	1.88
56	*Microcoleus* sp.	-	1	-	1	1	3	6	2.83
57	*Microcoleus lacustris*	-	2	-	1	-	2	5	2.35
58	*Scytonema ocellatum*	-	3	-	-	1	-	4	1.88
	Total	**35**	**110**	**21**	**72**	**22**	**76**	**336**	

S-1 = Baripada Block, S-2 = Suliapada Block, S-3 = Muruda Block
S = Summer Season (Rabi crop), W = Winter Season (Kharif crop)

Table 3. Diversity index of rice field cyanobacteria in different seasons and locations.

Location	Season	Total isolate	Species richness	Diversity index (Shannon –Wiener)
Site-1	Summer	35	26	3.184
	Winter	110	44	3.357
Site-2	Summer	21	17	2.610
	Winter	72	35	3.295
Site-3	Summer	22	19	2.878
	Winter	76	32	3.257

Site-1 = Baripada Block, Site-2 = Suliapada Block, Site-3 = Muruda Block
Summer Season = Rabi crop, Winter Season = Kharif crop

Table 4. Similarity coefficient of cyanobacteria in different locations and seasons.

Location	Season	Total isolate	Similarity Co-efficient					
			Site-1		Site-2		Site-3	
			Summer	Winter	Summer	Winter	Summer	Winter
Site-1	Summer	35	-	0.142	0.138	0.130	0.174	0.133
	Winter	110	-	-	0.090	0.125	0.108	0.118
Site-2	Summer	21	-	-	-	0.070	0.044	0.067
	Winter	72	-	-	-	-	0.105	0.119
Site-3	Summer	22	-	-	-	-	-	0.109
	Winter	76	-	-	-	-	-	-

Site-1 = Baripada Block, Site-2 = Suliapada Block, Site -3 = Muruda Block
Summer Season = Rabi crop, Winter Season = Kharif crop

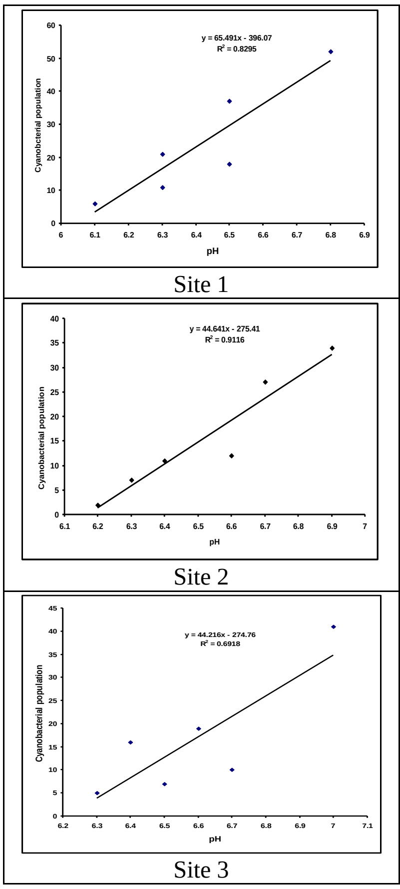

Fig. 1a. **Co-relation between cyanobacterial population and pH of different locations and seasons.**

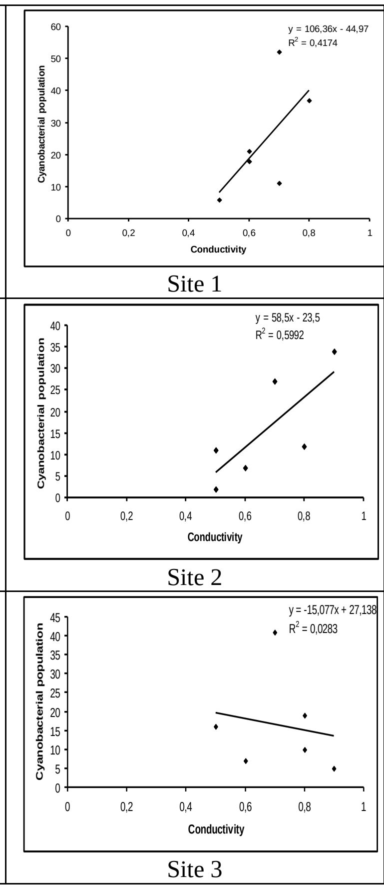

Fig. 1b. **Co-relation between cyanobacterial population and conductivity of different locations and seasons.**

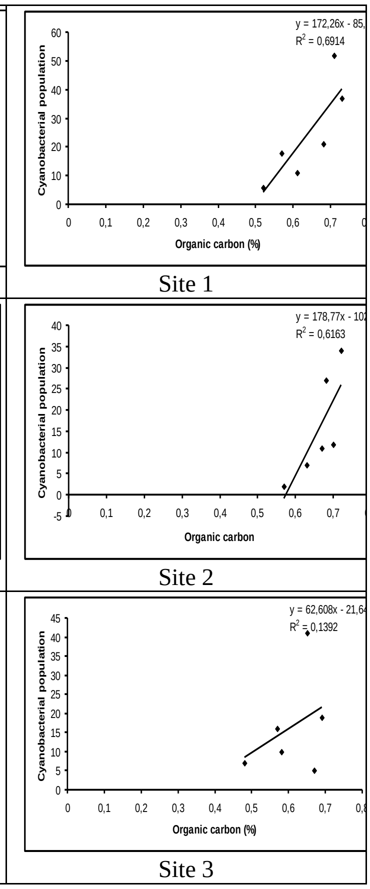

Fig. 1c. **Co-relation between cyanobacterial population and organic carbon (%) of different locations and seasons.**

DISCUSSION

Cyanobacteria, as natural biofertilizer, by maintaining the soil fertility increasing rice growth and yield is well established fact. In the present study rich diversity of cyanobacteria were recorded from local rice fields of Northern Orissa, India. The occurrence of cyanobacteria in the rice fields may be attributable to favourable environment with respect to their requirement for light, water, high temperature and nutrient availability, Which is in confirmation with the earlier finding (Konda and Yasuda 2003). The present investigation showed the predominance of non-heterocystous forms at all the locations. However, Nayak and Prasanna (2007) recorded more heterocystous forms while studying cyanobacterial abundance and diversity in rice field soils of India. Generally cyanobacteria form heterocysts during unfavorable environment and nutrient deficiency. The abundance of non-heterocystous forms indicates suitable environmental conditions for their growth. Nevertheless good number of heterocystous forms were observed during winter isolates which suggest the presence of some limiting factors in heterocyst development. In eastern India, winter is characterized by low temperature, water and light deficiency which may acts as limiting factors.

Similar assumption were made by several workers and reported predominance of heterocystous form during dry periods (Roger and Reynaud 1976, Roger and Kulasooriya 1980). The diversity of cyanobacteria in the rice fields were studied by Shannon index in two different seasons. Rich diversity was observed in all the study sites. Many workers have also reported both richness and evenness of cyanobacterial diversity from different rice fields of India (Prasanna *et al.* 2009). During winter the diversity index was more than summer and also both in the number of isolates and species richness. Few authors have reported seasonal variation of cyanobacteria in rice fields without elucidating on its diversity (Song *et al.* 2005). The rich diversity of cyanobacteria during winter in the present study may be attributed to favourable temperature and nutrient availability. Reports showed that blue-green algae are generally sensitive to high light intensities (Reynaud and Roger 1978) and may be regarded as low light species (Brown and Richardson 1968). Similar finding was made by Saadatnia and Riahi (2009) on studying cyanobacterial diversity from paddy fields of Iran. As mentioned earlier eastern India characterized by low temperature during winter however, in the present study (for winter) sampling was done mostly during October and November months, where the temperature is in the range 25 to 35°C which is reported optimum for growth of cyanobacteria.

Among different physico-chemical properties pH is important in determining growth, establishment and diversity of cyanobacterial flora, which is generally been reported to prefer neutral to slightly alkaline (Roger and Kulasooriya 1980, Kaushik 1994). In our study a high positive correlation is observed between the soil pH and cyanobacterial population, mostly non-heterocystous forms and contradicts that heterocystous forms were reported to be more abundant at alkaline pH (Nayak and Prasanna 2007). Although it is difficult to distinguish the effect of pH in determining algal flora composition that are due to other chemical factors, but it is obvious that it may rarely influence cyanobacterial population as negative correlation was observed for organic carbon (%) and conductivity in our present study. However, high diversity of heterocystous BGA of more than 95% has been reported even in dry season (Roger and Reynaurd 1976). Such finding indicates the ubiquitous distribution of cyanobacteria in natural environment. Several reports are available on the geographic regional distribution and the role of cyanobacteria in tropical rice fields (Singh 1961, Venkataraman 1981, Singh and Bisoyi 1989, Roger 1996), but their abundance and diversity vis-à-vis diverse rice ecologies is a relatively less explored area. In the present scenario, the use of cyanobacterial biofertilizers has much reduced mainly as a consequence of their poor establishment patterns in different soil types or ecologies. For wider exploitation and success of these biofertilizer technology in agriculture, coordinated strategic research efforts in the laboratory and at field level are highly essential.

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