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Diversity of Rice Field Cyanobacteria from Tropical Rice Field of Western Odisha

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Abstract: ***Diversity of Cyanobacteria from different tropical rice fields of WesternOdisha was studied.Eightysoil samples were collected from ten different sites.Sixty species of twenty one genera were isolated. Aulosirafertilissima and Nostoccarneumshowedhigh frequency of occurrence. Rice field soils of Chiplima and Rengali camp showed 18 different genera with 40 and 38 number of species respectively and Sason has poor Shannon's Diversity indexwith 15 species of 13 genera.***

Keywords: Cyanobacteria, Diversity, Rice fields, Western Odisha, Species richness

1. Introduction

India is a home of 1.21 billion people, i.e. ~17.4 % of total world's population and is represented as the 2nd most populated country in the world. However, it accounts for only 5.4 % of world GDP (Bhagwati, 2013).The economic position of the country is 10th in the world (Bloom, 2011).Sambalpur district lies between 20º43'N 22º11' N latitude and 82º39' E to85º13E longitude occupying an area of 6,667sq.km and the economy is cultivation based. It comprises 194,000 hectares of cultivable land (Odisha Agriculture Statistics, 2008-09).and out of 100 workers 53 are engaged in agricultural sector (Orissa Review, 2010 Dec.). Rice yield in tropical states like Odisha is affected due to some adverse factor like weed interaction, pest attack etc. and causing severe economic loss. Poor uneducated or less skilled farmers make their livelihood from agriculture and in order to increase rice yield they have used, pesticides (Sahu*et al*., 2015) and chemical fertilizer(NPK) in an unbalanced way(RCF,2013) leading to soil infertility.

Cyanobacteria act as the plough of nature since 3.5 GA (Olson and Blankenship 2004**,** Bhattacharyya *et al*., 2014) and now known to be the *nature's gadget* that manages the ecological balance of paddy-ecosystem through interactions among biophysical, biochemical and biodiversity milieus. Natural environment such as freshwater, hot springs, lakes, ponds, rivers and soil presents excellent habitats and favourable conditions for the luxuriant growth of this flora. The role of cyanobacteria in enhancing soil fertility by nitrogen fixation has been well documented long ago (Singh, 1961; Venkataraman, 1981). This well known autotrophic nitrogen engineer maintain NPK balance and C: N ratio of rice field through biological nitrogen fixation (Choudhary, 2011), Phosphate utilization and Photosynthesis. Apart from these cyanobacteria contribute to overall soil health by maintaining soil quality preventing erosion and production of bioactive compounds which have growth stimulating effect on plants.But due to modernization of agriculture i.e. with use of chemical fertilizer, pesticides etc (Bhattacharyya *et al*., 2011,2014) which graduallylead to losses of soil health. Massive industrialization also affects the cyanobacterial growth (Deep *et al*., 2013). Above factor may influence diversity and distribution of cyanobacteria in a region. Cyanobacteria are major representative of biodiversity that maintain the homeostasis of rice field as a sustainable system. Here in this study, an attempt has been taken to study the cyanobacterial diversity from different cultivated land of tropical rice fields of Sambalpur and Bargarh districts of Odisha, India

2. Methods

1) Study Site

The study was conducted fromdifferent rice fields of Sambalpur and Bargarh Districts of Western Odisha. Ten different sites were selected from different cultivated lands. These are Sason, Lapanga, Chiplima and Godbhagafrom Sambalpur district, Attabira,Lurupali,Rengali camp, Shuktapali andKalapani from Bargarh district and Kherual from Jharsuguda district.

2) Isolation and Enumeration of Cyanobacteria

Collected soil samples were air dried and homogenized and mixed together thoroughly. 1 g of soil sample of each site was kept in 5 petriplates with 40 ml of sterilized nitrogen free BG-11 medium under 7.5 W/m^2 light intensity at 25±2^{o}C in a culture room. After 10-12 days of incubation, algal colonies appeared on the plates, number of colony of each species were recorded (CFU), after observation under microscope. After about one week of growth, colonies appearing in agar plates were examined microscopically and data related to trichome shape, filament colour, akinete and heterocyst shape, size, position, number was recorded. Identification of cyanobacteria was done using the keys given by Desikachary (1959) and J Komarek (1998, 2005).

3. Data Analysis

a. Frequency of Occurance:

$$FO = \frac{Number\ of\ sample\ containing\ the\ species}{Total\ no\ sample\ examined} \times 100$$

b. Relative Frequency:

$$RF = \frac{Number\ of\ sample\ containing\ a\ species}{Total\ no\ of\ occurance\ of\ all\ the\ species} \times 100$$

c. Relative Density:

$$RD = \frac{Number\ of\ CFU\ of\ a\ species\ in\ all\ samples}{Total\ no\ of\ CFU\ all\ the\ species\ in\ all\ the\ samples} \times 100$$

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

$$RA = \frac{Number\ of\ samples\ containing\ the\ species}{Total\ no\ of\ occurance\ of\ all\ the\ species} \times 100$$

e. Diversity index: Cyanobacterial diversity in different sites has been calculated by Shannon's Diversity index (Shannon Wienner) as per the following formula

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

Where,
Hs- diversity in the sample S species or kinds
S- the Number of Species in the Sample
Pi- relative abundance of i^{th} species or Kind measures,n_i/N
N- total no of individuals of all kinds
n_i- no of individual of i^{th}species

4. Results and Discussion

Use of cyanobacteria as biofertiliser in improving soil fertility is well known. To use the full potential of cyanobacterial fertilizer to manage soil fertility synchronized efforts between laboratory and field research is required. Region-based specific cyanobacterial isolates could be more effective as they are pre-acclimatised to the existing environmental conditions. The composite mother culture of efficient nitrogen fixing strains collected from different locations of India and developed by National facility for blue-green algal collection at IARI, New Delhi are being cultured for their application as biofertiliser (Venkataraman, 1981). However, only few of these composite cultures could establish successfully in the fields of Orissa (Tripathy*et al*, 1990). Hence a region-specific biodiversity study is important for deriving optimum benefits from indigenous strains. Knowledge on cyanobacterial diversity of a region may help in selecting appropriate cyanobacterial inoculants to be applied as biofertilizer in crop fields as well as help in finding strains with other biotechnological potentials.

80 soil samples were collected from 10 independent sites, isolated cyanobacterial strains were observed under microscope and taxonomically important character such as shape of vegetative cell, heterocyst, akinete position of cyanobacterial strains were observed and identified with help of Desikachary (1959) and J Komarek (1998, 2005). Result shows Nostocalesare the dominating genera in the rice field of this region. Figure 1 shows the total CFU/gm of soil of the collected samples of the ten different places of Sambalpur and Bargarhdistricts. Data shows thatrice fields of Chiplima has highest CFU followed by Rengali camp.whereasSason and Kherualhas CFU less than 50%.Cyanobacteria are the major flora for revitalization of nitrogen economy of soil, thus lower the CFU, lower the rate of Nitrogen fixation i.e. soil fertility is at risk. Higher the CFU, higher the nitrogen fixation i.e. soil homeostasis is maintained. Thus rice field of Chiplima and Rengalicamp was found to be more cyanobacteria rich than Sason and Kherual.

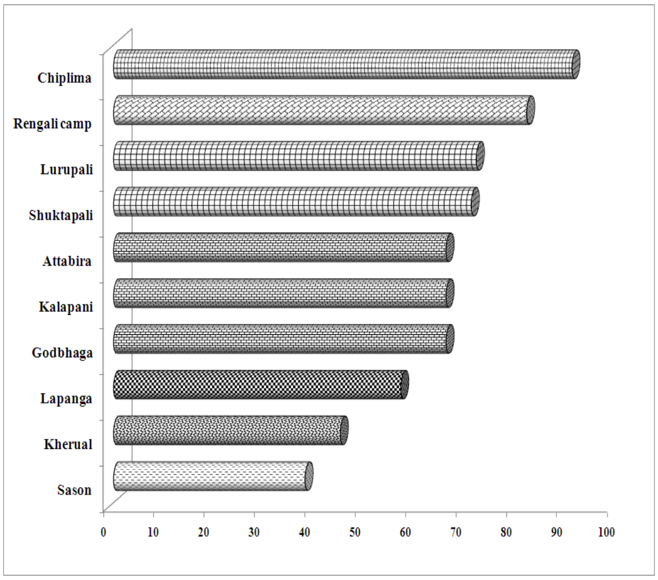

Figure 1: Species richness percentage of ten collection sites

60 species of 21 genera of cyanobacteria was isolated among which Frequency of Occurance (FO) of *Aulosirafertilissima* and *Nostoccarneum*is maximum (Table 1)followed by three species *Anabaena variabilis*, *Nostocpunctiforme* and *Nostocopsislobatus* of which FO is 90. Frequency of occurance of *Scytonema simplexis lowest* (10) *followed by Nostocpasserinianum* (20), *Anabaena ballygangii*, *Aphanothecesaxicola*, *Calothrixelenkinii*, *Gloeocapsa aeruginosa*, *Lyngbyabirgei*, *Nostocsphaericum*, *Oscillatoriaanguina*, *Oscillatoriachalybea*, *Oscillatoriaornate*, *Scytonema simplex*, *Oscillatoriacurviceps*, with FO is 30.Relative frequency of A. *ferilissima* and N. *carneum* is maximum i.e 3.24 within the study sites followed by A. *variabilis*, N. *punctiforme*, N. *lobatus* with RF value 2.91. Relative density of N. *lobatus* (5.33)and *Nostoccarneum* (3.96) is more than other cyanobacterial strains, Relative abundance is more in case of *N.lobatus*i.e 3.22. This data supports the finding of Nayak*et al* 2007. N. *lobatus*, A. *fertilissimia*, N. *carneum*, A. *variabilis*, N. *punctiforme*, are the dominating species of thetropical rice fields of this region.Many competent *Nostocsp* (Nilsson *et al* 2002), and *Anabaena sp.* (Adhikary, 2002) was able to colonize rice in root surfaces and intercellular spaces having higher nitrogenase activity compared to their free-living species.

Table 1: Relative frequency(RF)Relative density(RD), Relative abundance(RA), and Frequency of occurrence (FO) of isolated cyanobacteria collected from rice field

Sl No	*Name of species*	*FO*	*RF*	*RD*	*RA*
1	*Aulosirafertilissima*	100	3.24	3.81	2.07
2	*Nostoccarneum*	100	3.24	3.96	2.15
3	*Anabaena variabilis*	90	2.91	3.50	2.12
4	*Nostocpunctiforme*	90	2.91	3.50	2.12
5	*Nostocopsislobatus*	90	2.91	5.33	3.22
6	*Anabaena fertilissima*	80	2.59	2.89	1.97
7	*Calothrixbraunii*	80	2.59	2.13	1.45
8	*Oscillatoriachlorina*	80	2.59	1.83	1.24
9	*Anabaena oryzae*	70	2.27	3.04	2.37

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10	*Anabaena sphaerica*	70	2.27	2.89	2.25
11	*Anabaena torulosa*	70	2.27	2.13	1.66
12	*Calothrixparietina*	70	2.27	1.52	1.18
13	*Lyngbyaspiralis*	70	2.27	3.81	2.96
14	*Nostocellipsosporum*	70	2.27	3.35	2.60
15	*Phormidiumjadinianum*	70	2.27	1.98	1.54
16	*Aphanothecestagnina*	60	1.94	1.83	1.66
17	*Aulosiraprolifica*	60	1.94	3.04	2.76
18	*Aulosirasp*	60	1.94	1.37	1.24
19	*Lyngbyaconfervoides*	60	1.94	1.98	1.79
20	*Nostoccalcicola*	60	1.94	1.67	1.52
21	*Rivulariaaquatica*	60	1.94	1.83	1.66
22	*Scytonemasp*	60	1.94	0.91	0.83
23	*Cylindrospermummusicola*	50	1.62	1.22	1.32
24	*Cylindrospermum sp.*	50	1.62	1.67	1.82
25	*Gloeocapsapunctata*	50	1.62	1.98	2.15
26	*Microchaetesp*	50	1.62	2.28	2.48
27	*Microcystiselabens*	50	1.62	0.91	0.99
28	*Microcystis aeruginosa*	50	1.62	1.37	1.49
29	*Nostoc commune*	50	1.62	1.37	1.49
30	*Oscillatoriasalina*	50	1.62	2.13	2.32
31	*Oscillatoria tenuis*	50	1.62	1.52	1.66
32	*Rivulariasp*	50	1.62	1.37	1.49
33	*Anabaena cylindrica*	40	1.29	1.22	1.66
34	*Anabaena circinalis*	40	1.29	0.91	1.24
35	*Aphanocapsabanaresensis*	40	1.29	1.07	1.45
36	*Calothrixmarchica*	40	1.29	0.76	1.04
37	*Chroococcuspallidus*	40	1.29	1.07	1.45
38	*Chroococcusturgidus*	40	1.29	1.22	1.66
39	*Desmonostocmuscorum*	40	1.29	2.13	2.90
40	*Gloeocapsakuetzingiana*	40	1.29	0.76	1.04
41	*Haplosiphonsp*	40	1.29	1.07	1.45
42	*Nostocrivulare*	40	1.29	1.98	2.69
43	*Oscillatoriaprinceps*	40	1.29	0.76	1.04
44	*Oscillatoriapseudogeminata*	40	1.29	0.91	1.24
45	*Phormidiummucosum*	40	1.29	0.76	1.04
46	*Tolypothrixfragilis*	40	1.29	0.61	0.83
47	*Westiellopsisprolifica*	40	1.29	0.91	1.24
48	*Westiellopsissp*	40	1.29	0.61	0.83
49	*Anabaena ballygangii*(banerji)	30	0.97	0.46	0.83
50	*Aphanothecesaxicola*	30	0.97	0.61	1.10
51	*Calothrixelenkinii*	30	0.97	0.61	1.10
52	*Gloeocapsa aeruginosa*	30	0.97	0.61	1.10
53	*Lyngbyabirgei*	30	0.97	1.67	3.04
54	*Nostocsphaericum*	30	0.97	1.07	1.93
55	*Oscillatoriaanguina*	30	0.97	0.91	1.66
56	*Oscillatoriachalybea*	30	0.97	1.22	2.21
57	*Oscillatoriaornata*	30	0.97	0.76	1.38
58	*Oscillatoriacurviceps*	30	0.97	0.46	0.83
59	*Nostocpasserinianum*	20	0.65	0.61	1.66
60	*Scytonema simplex*	10	0.32	0.15	0.83

Species richness of rice field soil (Figure 2) of Chiplima is highest (13.03%) followed by Rengali camp (12.38%), Lurupali(11.40%) and Kalapani(10.75) where as Kherual (7.46%) and Sason(5.08%) shows lowest species richness.

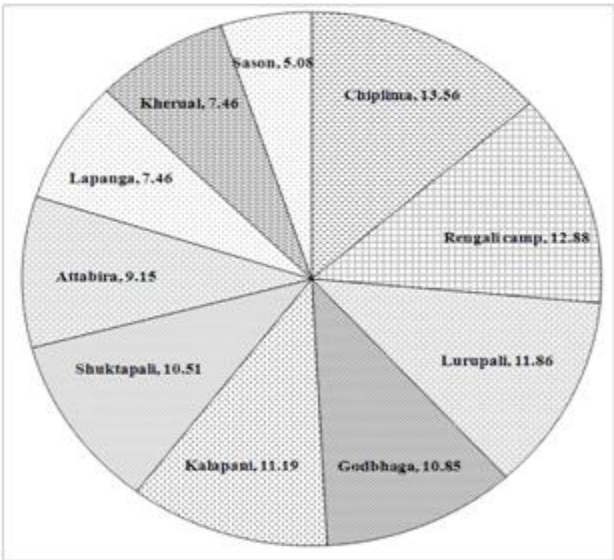

Figure 2: Species richness percentage of ten collection sites

Diversity index of cyanobacterial population in the Rice field of different sites of Sambalpur were calculated by Shannon-Wienner Method (Table2). Among the locations Chiplima and Rengali camp has relatively higher diversity index. 18 different genus of cyanobacterial species were found from rice fields soil of Chiplima and Rengali camp with 40 and 38 number of species respectively and Sason has poor diversity index with 13 genera with 15 species. Lower in diversity index may be due to extensive use of pesticides(Adhikary 2002) and chemical fertiliser, presence of toxic substances in the soil. From our earlier study (Deep *et al.*, 2013) we found that massive industrialisation adversely affects the wetland cyanobacterial flora. Irrigation through polluted water bodies or due to settlement of flyash in the rice field of Sason, Kherual and Lapanga minimised the growth of cyanobacteria. Thus most of the field has minimum production where as rice field of Chiplima, Rengali camp, Kalapani, Lurupali shows more species richness and diversity index which leads to increase in soil fertility.*Anabaena fertilissima*, *Anabaena oryzae*, *Anabaena variabilis*, *Aulosirafertilissima*, *Aulosiraprolifica*, *Desmonostocmuscorum*, *Lyngbyaspiralis*, *Nostoccarneum*, *Nostocellipsosporum*, *Nostocpunctiforme*, *Nostocrivulare* and *Nostocopsislobatus*has higher FO, RD, RA value. An extensive study is needed using these organism as inoculums for their use as biofertiliser as they are pre-acclimatised in all the collected sites.

Table 2: Occurrence and distribution of cyanobacteria in various locations of Sambalpur, India along with Shannon-Wienner diversity index (H).

Collection Sites	No of Genus	Species Richness	Hs
Chiplima	18	40	3.60
Rengali camp	18	38	3.52
Lurupali	19	35	3.41
Godbhaga	14	32	3.36
Kalapani	18	33	3.34
Shuktapali	15	31	3.24
Attabira	11	27	3.08
Lapanga	15	22	2.11
Kherual	16	22	2.02
Sason	13	15	1.8

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