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Influence of Physico-Chemical Factors on Heterocytous Cyanobacterial Diversity Thrive in the Rice Fields of Western Odisha

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ABSTRACT

The Western part of Odisha is agriculturally developed because of the presence of two mighty river Mahanadi and Brahmani and maximum rice cultivation in the Western part compared to other parts of Odisha. Cyanobacteria are one of the dominant groups of microorganisms present in the rice field soil. These are a very potential group of bacteria that enrich the soil fertility by biological nitrogen fixation because conducive waterlogging condition helps their luxurious growth. However, the native cyanobacterial diversity and physico-chemical properties of rice fields of Western Odisha poorly studied. Hence, a tenacious study has been conducted on indigenous cyanobacterial diversity, their distribution pattern, the influence of physico-chemical factors on diversity indices of rice fields of the Western part of Odisha. A total of 25 heterocytous cyanobacterial isolates belonging to four genera, namely, *Nostoc, Anabaena, Calothrix* and *Hapalosiphon* were documented. Among the physico-chemical properties, soil pH was slightly acidic, a high range of organic carbon was found, total nitrogen, phosphorus and potash were found in a wide range. The maximum Shannon-Wiener index was observed in the Kherual rice field. The study highlighted that *Nostoc* is the most dominant genus among all genera studied. It was found that cyanobacterial diversity was more in rice fields of Kherual areas compared to rice fields of other six areas. Further findings of this study indicated thatthe variation of soil physico-chemical factors of rice fields of different locations did not play a hindrance to the distribution of these cyanobacterial genera.

Keywords Heterocytous cyanobacteria, Physico-chemical factors, Shannon-Wiener index, Rice fields.

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INTRODUCTION

The state Odisha lies under the tropical belt of the Eastern region of India. The Western part of Odisha is mostly mountainous and hilly. Due to the presence of two major rivers, i.e., Mahanadi and Brahmani, these areas are agriculturally developed. In this region, broadly four types of soil are found, namely, red soil, mixed red and yellow soil, black and laterite soil. The climate of this region experience extreme type with hot and dry summer, humid monsoon and ruthless cold weather. The temperature of this part of Odisha varies between 10 °C to 45 °C (Gouda et al. 2017). The presence of different soil types and variable climatic conditions provide a suitable habitat for the growth of a wide range of soil micro-organisms (Morris and Blackwood 2015). Cyanobacteria are photosynthetic oxygenic nitrogen-fixing bacteria that play a crucial role in the maintenance of soil fertility in rice fields. During rice cultivation, there isa huge growth of cyanobacteria observed because of waterlogging conditions of rice fields (Whitton 2000). These bacteria are excellent nitrogen fixer because they possess nif gene and heterocytes as specialized cells for nitrogen fixation. Besides, they have various beneficial properties such as promoting plant growth regulators, increasing phosphate content of the soil, which helps wasteland reclamation and climate change mitigation through CO_2 sequestration and many more (Singh etal. 2016). Good numbers of literature have been published on cyanobacterial diversity in the rice fields of different parts of India (De 1936, Mitra 1951, Singh 1961, Aiyer 1965, Pandey 1965a, Patnaik 1966, Tiwari et al. 2001). They are broadly classified into two categories such as nitrogen and non-nitrogen fixing cyanobacteria. The National Rice Research Institute, Odisha, reported that the heterocytous cyanobacteria, namely, *Nostoc, Anabaena, Rivularia, Tolipothrix, Calothrix, Aulosira, Westiellopsis* and *Cylindrospermum* were dominant in rice fields. Moreover, there is a large variation in the cyanobacterial diversity and distribution in the rice fields. This variation may depend upon the physico-chemical property of soil in the rice fields (Sahu et al. 1996, Nayak and Prasanna 2007). Among the physico-chemical properties of soil, pH plays an important role in cyanobacterial growth and diversity. Although cyanobacteria often considered as soil conditioners and change the pH of the soil but neutral to slightly alkaline pH shows the optimum pH in their growth (Singh 1961). Macronutrients such as nitrogen and phosphate deficiency do not affect the growth of cyanobacteria. The dominance of cyanobacteria enhances the phosphate and nitrogen content of the soil (Zancan et al. 2006). Generally, a moderate-to-high range of potassium increase the cyanobacterial abundance in rice fields (Roger and Kulasooriya 1980). Our knowledge of the native cyanobacterial populations in the rice fields of Western Odisha quite meager.Therefore, the main purpose of this investigation was to estimate the diversity of heterocytous cyanobacteria, their relative abundance and the influence of physico-chemical factors of soils on cyanobacterial diversity and species distribution of rice fields of Western Odisha.

MATERIALS AND METHODS

Sampling site

The samples were collected from seven sites (S_1 to S_7) in or around Sambalpur district, Odisha, namely, Lapanga (21.7077° N, 84.0057° E), Hikudi (20.6278° N, 83.2667° E), Kherual (21.7926° N, 84.0177° E), Nuagaon (21.6789° N, 84.386° E), Sason main canal (21.4241° N, 84.0795° E), Rengali (21.6339° N, 84.0426° E) and Badmal (21.816° N, 84.0086° E). Isolation and purification of cyanobacterial strains One gram of the aliquot soil sample was measured and dissolved in distilled water in different glass tubes separately and the serial dilution method was performed. After serial dilution, the diluted samples were inoculated into BG11 medium in agar plates without nitrogen supplement (Rippka et al. 1979). These agar plates were placed under continuous white fluorescent illumination (60 µmol m^{-2} s^{-1}) at 25 ± 2 °C for 2–3 weeks. Repeated sub-culture was performed to obtain pure strains by following standard methods (Andersen and Kawachi 2005).

Morphological analysis and identification

Cyanobacterial species isolated from the collected samples were observed regularly under a microscope and important morphological characteristics were noted. The microphotographs of each pure strains were taken using a phase-contrast microscope (Zeiss Axiostar Plus, Carl Zeiss). The taxonomic identification was based on important morphological characteristics of the strains followed by monographs devised by Desikachary (1959), Komárek (2013).

Physico-chemicalproperties of soil

The physico-chemical properties include temperature, pH, total organic carbon, nitrogen, phosphorus and

Table 1. Cyanobacterial species distribution in rice field of western Odisha. S_1 (Lapanga), S_2 (Hikudi), S_3 (Kherual), S_4 (Nuagaon), S_5 (Sason), S_6 (Rengali), S_7 (Badmal), + (percent) and - (absence).

Sl. No.	Cyanobacterial isolates	S1	S2	S3	S4	S5	S6	S7	RF (%)	FO (%)
1.	*Calothrix javanica* isolate: 4	-	+	-	-	+	-	-	3.389	28.5
2.	*Calothrix marchica* isolate: 5	-	-	-	+	+	+	-	6.779	57.14
3.	*Calothrix* sp. 8-SKD-2014	-	-	-	-	-	-	+	1.694	14.28
4.	*Calothrix* sp. 8-SKD-2014	+	-	-	+	-	+	-	5.084	42.85
5.	*Calothrix* sp. 13-SKD-2014	-	+	-	-	-	-	-	1.694	14.28
6.	*Calothrixweberii* 22-SKD-214	+	-	-	+	-	+	-	5.084	42.85
7.	*Hypalosiphonwelwitschii* 7-SKD-2014	-	+	-	-	+	-	+	5.084	42.85
8.	*Anabaena variabilis* isolate 10-SKD-2014	+	-	+	-	-	-	-	3.389	28.57
9.	*Anabaena variabilis* isolate: 17-SKD-2014	-	+	-	+	-	-	+	5.084	42.85
10.	*Anabaena iyengarii* isolate18-SKD-2014	+	-	+	-	-	-	-	3.389	28.57
11.	*Anabaena orientalis* isolate: 20-SKD-2014	-	+	-	-	-	-	+	3.389	28.57
12.	*Anabaena iorulosa* isolate: 23-SKD-2014	-	+	-	-	-	-	-	1.694	14.28
13.	*Nostac carneum* isolate:1	+	-	+	-	-	-	-	3.389	28.57
14.	*Nostac elipsosporum* siolate: 3	+	-	+	-	-	-	-	3.389	28.57
15.	*Nostac punctiforme* isolate: 6SKD-2014	+	-	-	+	-	+	-	5.084	42.85
16.	*Nostocparmelioides* 11--SKD-2014	+	-	+	+	-	-	-	3.389	28.57
17.	*Nostoc* sp.14-SKD-2014	-	-	-	+	+	+	-	5.084	42.85
18.	*Nostoc* sp. 16-SKD-2014	+	-	+	-	-	-	-	3.389	28.57
19.	*Nostoc oryza* 19-SKD-2014	-	-	-	-	+	+	-	3.389	28.57
20.	*Desmonosstoc muscorum* isolate 21-SKD-2014.	-	-	+	-	+	+	+	5.084	42.85
21.	*Nostoc calcicola* isolate: 24-24-SKD-2014	-	-	+	+	+	+	-	6.779	57.14
22.	*Nostoc* sp. 25-SKD-2014	+	-	+	-	-	-	-	3.389	28.57
23.	*Nostoc* sp. 26-SKD-2014	+	-	+	-	-	-	-	3.389	28.57
24.	*Nostoc oryza* 28-SKD-2014	-	-	-	-	-	+	-	1.694	14.28
25	*Nostoc oryza* 29-SKD-2014	-	-	-	-	+	+	-	3.389	28.57
	Total individual type of cyanobacterial isolates	10	7	9	8	9	10	7		

potash were analyzed. Soil pH was measured from 1: 5 (soil: distilled water) of air-dried soil samples. Soil pH was measured using a pH meter (Systronic 324). Soil temperature was directly measured from the field using a thermometer, while sample collection was processed. Total organic carbon was estimated according to Nelson and Sommers (1996). To estimate potash, the ammonium acetate extraction method was followed by (Volk and Troug 1934). The phosphate content of the soil was estimated by the developed protocol (Dickman and Bray 1940). Total soil nitrogen was analyzed by the Kjeldahl method using the Pelican auto-analyzer. (Bradstreet 1954).

Diversity data analysis

A. Relative Frequency (RF):

$$RF = \frac{Y}{X} \times 100$$

(Y: Number of soil samples containing a species, X: Total number of occurrences of all cyanobacterial isolates)

B: Frequency of Occurrence (FO):

$$FO = \frac{Y}{X} \times 100$$

(Y: Number of Soil samples containing a cyanobacterial isolate, X: Total number of soil samples examined)

C: Diversity Index: Cyanobacterial diversity in different sites was calculated using Shannon-Wiener index (Hs) according to the formula

$$HS = -\sum_{i=1}^{S} (P_i)\ (\ln P_i)$$

Where Pi= the relative abundance of cyanobacterial isolate calculated by the following equation

$$= \frac{n}{N}$$

n: total number of cyanobacteria of particular species, N: Total number of cyanobacteria of all species

D : Simpson's index of dominance (D) : $\sum Pi^2$

Results and discussion

A total of 25 heterocytous cyanobacterial species were isolated and identified. These cyanobacterial species mainly belong to two orders i.e., Nostocales and *Stigonematales* of subsection IV and subsection V, respectively, described in Bergey's manual of systemic bacteriology. These identified cyanobacterial species were belonging to four genera namely, *Calothrix, Hapalosiphon, Nostoc*, and *Anabaena.* Cyanobacterial distribution data of Western Odisha rice fields showed that the species richness was in the order of *Nostoc, Calothrix, Anabaena,* and *Hapalosiphon* (Table 1).The findings agreed with a previous report of Nayak and Prasanna 2007. The total number of occurrence of cyanobacterial isolates from all locations were 59. A quantitative occurrence wise maximum of 10 isolates was obtained in Lapanga and Rengali rice fields. Among the frequency of occurrence of heterocytous cyanobacteria, *Calothrix marchica* isolate: 5 and *Nostoc calcicola* isolate: 24-SKD-2014 was widely distributed in different regions. Similarly, the relative frequency was found to a maximum for Calothrix marchica isolate: 5 and Nostoc calcicola isolate: 24-SKD-2014 and lowest in *Calothrix* sp. 13-SKD-2014, *Anabaena torulosa isolate:* 23-SKD-2014.

Rice field soils of 7 locations were used for physico-chemical analysis. The results of temperature, pH, organic carbon, phosphorus, potassium, and total nitrogen parameters were analysed (Table 2). The soil samples were collected during March-April, the month of 2012. During that period the maximum atmospheric temperature was in a range of 38 °C to 40 °C in the Western part of Odisha. The maximum soil temperature was found in Rengali and Kherual division; the lowest value was 36 °C in the Badamal region. This is the reason, we did not observe a large variation in soil temperature. In the present investigation, pH values indicate slightly acidic in the range of 5.98 ± 0.21 to 6.61 ± 0.22 in soil samples. The reason behind the decrease in soil pH may be intensive cultivation (Rudramurthy et al. 2006) and the addition of synthetic fertilisers (Lin et al. 2019). The maximum number of cyanobacterial isolates found in Rengali and Lapanga was relatively high pH followed by Kherual has the lowest pH. This shows the range of pH at which indigenous cyanobacterial diversity and their abundance in soil inhabit. Organic carbon (C) percentage was varied from 1.42 ± 0.1 to 4.95±0.04. It was found that the C percentage was very high in all sampling sites and Nuagaon and Lapanga had maximum and minimum content, respectively. Soil organic carbon content is closely linked to soil

Table 2. Phjysico-chemical properties of soil samples from rice fields of different parts of Western Odisha.

Lucations	Longitude & Latitude	Temp (^{0}C)	pH	C (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Lapanga	21.7077^{0}N, 84.0057^{0}E	38.2±0.2	6.46±0.15	1.42±0.1	350.0±3.4	57.0±2.3	210.0±2.6
Hikudi	20.6278^{0}N, 83.2667^{0}E	36.5±0.2	6.5±0.14	1.85±0.03	383.0±4.4	59.0±1.3	540.0±5.1
Kherual	21.7926^{0}N, 84.0177^{0}E	38.5±0.1	5.98±0.21	3.70±0.06	399.0±4.8	3.0±0.2	400.0±4.5
Nuagaon	21.6789^{0}N, 84.386^{0}E	37.0±0.3	6.12±0.06	4.9±0.04	669.0±2.1	23.0±0.8	290.0±3.6
Sason	21.4241^{0}N, 84.0795^{0}E	36.5±0.1	6.32±0.07	3.27±0.03	650.0±5.0	28.0±2.2	600.0±6.8
Bengali	21.6339^{0}N, 84.0426^{0}E	38.5±0.2	6.61±0.22	2.85±0.05	255.0±7.1	14.0±1.1	130.0±5.2
Badamal	21.816^{0}N, 84.0086^{0}E	36.0±0.4	6.05±0.34	2.70±0.13	648.0±6.0	3.0±0.5	530.0±4.1

structure, improve soil fertility and favours soil-water relation (Xing et al. 2004). After the rice harvesting season, excess straws leftover the soil might have caused a very high carbon percentage in the fields under study. The results show a positive correlation between the number of cyanobacterial isolates and total organic carbon content. An earlier report also agrees with the fact that organic carbon plays a major role in controlling the growth and distribution of cyanobacteria (Ibraheem 2003). It was found that nitrogen content in all rice fields ranged from 255.0 ±7.1 to 669.0±2.1 kg ha^{-1} and lowest content found in Nuagaon and Lapanga. Further, the cyanobacterial abundance was noted maximum at medium to high range of nitrogen. This suggests that the availability of nutrients like nitrogen is favourable for cyanobacterial presence (Zancan et al. 2006). In other-way around, another possibility of high concentrations of nitrogen may be due to the presence of nitrogen-fixing cyanobacteria (Nascimento et al. 2019). The phosphorus content indicated a significant difference between the rice fields. The highest level of phosphorus was 59.0 ± 1.3 Kg ha^{-1} reported from the soil samples collected from Hikudi. In a previous study reported that phosphorous content in the soil samples is one of the major nutrients regulating plant productivity (Drink-water and Snapp 2007). Our finding proposes that cyanobacterial dominance does not necessarily depend on the soil phosphorous content; instead, they are distributed in all rice fields with a wide range of phosphorous content. Cation like potassium showed a significant difference between the soil samples. In this study, the potassium content ranged between 130.0 ± 5.2 to 600.0 ± 6.8 Kg ha^{-1} in the rice fields. The site having the lowest potassium was Rengali and Sason's main canal found to have maximum. According to Pankratova (2006), in a temperate environment, the presence of phosphorous and potassium directly influences algal dominance. Our finding in line with the previous report that the maximum number of occurrences of cyanobacteria found in a very high concentration of potassium.

The diversity index of cyanobacterial isolates occurring in various rice fields was calculated using the Shannon-Wiener (Hs) method and Simpson's index of dominance (D) listed (Table 3). Here, *Nostoc* and *Calothrix* were widely distributed in all sites, which were observed from their species richness data. Shannon-Wiener index, which reflects species richness and relative abundance of each of these two species in the sampling area showed a maximum in the rice fields of Kherual (0.786) followed by Rengali. Maximum Simpson's index was recorded from Badamal (1.326) and the lowest from Kherual (0.354). These two locations reflect the most stable environment among the experimented fields with a favourable climate for rice cultivation and create a suitable condition for cyanobacterial growth and diversity

Table 3. Diversity index of rice field cyanobacteria in different location.

Locations	Total cyanobacterial isolates	Species Richness	Shannon – Wiener index	Simpson indes of dominance (D)
Lapanga	10	5	0.46	0.89
Hikudi	7	3	0.427	0.953
Kherual	9	2	0.786	0.254
Nuagaon	8	3	0.343	1.08
Sason	9	4	0.375	1.147
Rengali	10	2	0.58	0.61
Badmal	6	4	0.272	1.326

The relationship between the distribution of genera with the response to the physico-chemical properties of the rice fields was analysed (Table 4). We found that almost half of the isolates were falling under the temperature below (31 occurrences) and above (28 occurrences) 38 °C. Again a maximum number of *Nostoc* species was found to grow above 38 °C in comparison to *Anabaena isolates*. Among all sites and a maximum number of Nostoc followed by *Calothrix i*solated from the soil sample. It was shown that the occurrence of *Nostoc* was relatively high in comparison to other genera in the higher temperature area. This finding was in agreement with an earlier study observed (Gupta and Agrawal 2006). In the previous study, Chen et al. (2011) reported that soil cyanobacteria like *Nostoc flagelliforme* can grow at temperatures up to 65 C. Further, Nisha et al. (2007) reported that indigenous cyanobacteria have the potential to tolerate harsh conditions such as dry weather and high temperature. Therefore, from the present study, it may be attributed that cyanobacterial species such as *Nostoc, Calothrix, Anabaena,* and *Hapalosiphon* can grow at high temperatures but up to a limit.

Table 4. Distribution of cyanobacterial genera concerning the physico-chemical factors. VH (Very high), H (high), M (Medium, L (Low), VL (Very low).

Cyanobacterial genera	Temperature (^{0}C)		C (%)		N (kg ha$^{-1)}$			P (kg ha^{-1})				K (kg ha^{-1})		
	<38	>38	VH	H	H	M	L	H	M	L	VL	VH	H	M
Anabaena	09	02	11	0	05	03	03	05	00	03	03	08	02	01
Nostoc	11	20	26	0	10	17	04	06	11	04	10	15	03	13
Calothrix	08	06	14	0	06	05	01	05	05	03	01	05	03	06
Hapalosiphon	03	00	03	0	02	01	0	01	01	0	01	03	0	0
Total no of individual cyanobacterial species isolated from different location	31	28	59	0	23	26	10	17	17	10	15	31	08	20

Further more, all the cyanobacterial strains were isolated from high carbon content areas. The maximum number of isolates of the *Nostoc* genus were retrieved from soil samples, having medium-ranged of nitrogen content. The phosphorus content was found to be the most variable macronutrient among the soil samples being studied. It showed that the maximum number of cyanobacterial isolates shared the soil samples having high and medium-ranged phosphorus. Most of the cyanobacterium was found in very high content followed by a medium level of potassium. Physico-chemical properties of two locations Kherual and Rengali indicated that the wide range of variation and the maximum diversity obtained from these two locations. The above findings supported by Simpson's dominance index suggesting greater the value lower the diversity and vice versa shows Kherual has the lowest and Badmal has maximum.

From the above results, we may infer that physico-chemical factors of the rice field soils have a greater role in heterocytous cyanobacterial distribution but it is unclear what would be the minimum and maximum nutrient content that will regress or stimulate cyanobacterial diversity. Further, the pH, soil temperature, organic carbon, nitrogen, phosphorous and potassium content of the experimental rice field soil possibly not acted as restrictive factors for cyanobacterial richness and dominance; the cause behind the lower diversity and lower species richness may co-relate with the use of various agrochemicals in the rice fields.

CONCLUSION

The present study investigated the physic- chemicals and heterocytous cyanobacterial diversity of rice fields soil of Western Odisha. It was concluded from clearly elicited results of the present study that the rice field soils of Western Odisha are slightly acidic, contained a high percentage of organic carbon and wide range of N, P, K. Moreover, these factors congruently influence indigenous cyanobacterial diversity of rice fields of these areas but not the control factors for a change of species richness and dominance. Further, It was concluded that the cyanobacterial diversity of Western Odisha rice fields was dominated by Nostoc followed by Calothrix, Anabaena, and Hapalosiphon. Further, maximum cyanobacterial diversity recorded from rice fields of Kherual and with the least number of species richness and Badmal has the highest number of species richness and minimum cyanobacterial diversity.

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