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NITROGEN FIXING CYANOBACTERIAL FLORA OF NADIA DISTRICT IN WESTBENGAL

N. C. Halder

Department of Botany, Uluberia College, under the University of Calcutta, Uluberia, Howrah 711 315, West Bengal, India.

Abstract

Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increase rice growth and yield as a natural bio fertilizer. The paddy field ecosystem provides a favourable environment for the growth of cyanobacteria with respect to their requirement for light, water, temperature and nutrient availability. Continued three successive years (2011-2014) studied the occurrence of cyanobacteria in rice growing fields of Nadia district in West Bengal, India. We survey and studied 3 different rice growing fields of Nadia district namely Haringhata, Kalayani and Jaguliya for studying the cyanobacterial diversity. These rice growing fields mainly comprises unicellular, non-heterocystous and heterocystous cyanobacteria. Total 37 species of Nitrogen fixing cyanobacteria samples belonging to 21 genera and four orders i.e., Chroococcales, Oscillatoriales, Nostocales, and Stigonematales were isolated from various rice growing localities. Present investigations revealed that the pH range of soil was mostly acidic in Rabi season and it was more than Kharif crop season. Three major cyanobacterial groups; Unicellular and colonial were slight more than Non-heterocystous i.e., 6 genera and 8 species. Non-heterocystous filamentous cyanobacteria were minimum and its 5 genera and 8 species and heterocystous filamentous cyanobacteria were maximum, its 10 genera and 19 species were occurrence in most of all low and upland rice growing areas.

Key words: Chroococcales, Cyanobacteria, Nostocales, Oscillatoriales, Stigonematales.

Introduction

The use of cyanobacteria (BGA) as biofertilizer of rice crop was first reported by P. K. De (1939). Cyanobacteria possess an autotrophic mode of growth like eukaryotic plant cells, metabolic system like bacteria and occupy a unique position. They exhibit a great morphological diversity and their broad spectrum of physiological properties reflects their widespread distribution and tolerance to environmental stress (Tandeau de Marsac and Howard, 1993). Cyanobacteria is a nitrogen fixing potential of diazotrophic microorganism, which is of great significance for enriching of nitrogen level in soil. Debnath and Ray et al., 2009; Keshri and Chatterjee et al., 2010; Zhang and Zhang et al., 2010 observed some problem for the cyanobacterial distribution and its peculiarity to proper identification. The cyanobacterial biofertilizer technology is well proven but still it is facing problem of availability of proper

strains as starter cultures. Therefore, it is very essential to undertake extensive survey to rice fields to explore the status of cyanobacteria flora especially nitrogen fixing species along with ecology and physiochemical properties of different rice growing fields. In respect to their role in increasing the fertility of rice soils cyanobacteria are of the special academic and applied interest. We survey and studied the distribution of blue green algal biodiversity of three different sites i.e., Haringhata, Kalyani and Jaguliya and their adjoining areas of Nadia district in West Bengal.

Materials and Methods

Isolation of the culture

Cyanobacteria samples along with soil and water were collected from major rice growing three different sites i.e., Haringhata, Kalyani and Jaguliya and their adjoining areas of Nadia district. Collection were made during Kharif crop season i.e., July to October and in Rabi i.e., November to June from 2011 - 2014 and samples were isolated under investigation. During the collection, prominently visible growths of blue green algae were collected in plastic and polythene containers for direct observation with the help of MOTIC Trino-oculer microscope imaging system and the heterogeneous suspension was diluted with the liquid culture BG11 Medium (Stanier et al., 1971).

Selection of media

In the beginning Bold's Basal Medium (Bischoff and Bold, 1963), Chu No 10 (CHU, 1942) and BG11 Medium (Stanier et al., 1971) have been used. Among these, BG11 medium supported the better growth of the cultures. The final pH was adjusted at 7.5. During the study, all the experiments performed in BG11 medium (liquid and solid, with or without Nitrate nitrogen) and strains were maintained in stock cultures in this medium with in sterile soils and incubated for 25-30 days at 30^{0}C ± 2 and 3000-3500 Lux light intensity under 14/10 LD cycle.

Maintain of Cyanobacterial strains

All the isolated strains of Cyanobacteria are being maintained in culture collection in the Department of Botany, Uluberia College.

Physicochemical Properties of Soil

Different parameters i.e., soil temperature, pH, EC (Electric Conductivity), N, P, K and Organic carbon were taken into consideration for the measurement of fertility levels in soils. The available soil phosphate, potassium, total soil nitrogen and organic carbon were estimated as per procedures described by Trivedi and Goel, 1986 in the laboratory. The soil temperature, conductivity and pH were determined by using soil thermometer, conductivity meter and digital pH meter respectively.

Identification of the isolates

The identification of the selected isolates for morphological and physiological studies have been made using standard monographs of Geitler, 1932; Desikachary, 1959; Starmach, 1966; Komarek and Anagnostidis, 1986, 1988 and our present observations. The observations made on all the isolates are described below only with the occurrence, nature of the thallus, sheath, cells, heterocytes, development of hormogons, benching pattern, akinete, spores.

Results and Discussion

Occurrence of Blue-green algae in rice growing fields of Nadia district, West Bengal has been under taken for the first time in this area. Nadia is one of the best rice growing districts due to its soil classes, pattern of rain fall, and temperature and weather variability in India. It is situated between 22^{0}53" and 24^{0}11" North latitude and 88^{0}09" and 88^{0}48"

East longitude, and covers area about 3927 sq. kms in West Bengal.

Detailed survey have been made and collected data are given below

Geographical Location

Total area of Nadia district: **3,927km^2 ,**

Latitude-20^{0}53'N-24^{0}11'N and

Longitude- 88^{0}09'E – 88^{0}48'E.

Character of Soil

Soil Color - Grayish to blackish.

Soil Type - Sandy to loamy and clay.

Weather Record - Average Rainfall - June to September - 269 mm ± 5;

Average Temperature – Summer - (26±2 to 41±1)^{0}C, Winter - (8 - 26± 1)^{0}C

Average Range Humidity (%) - July to September- 67 to 89% ± 2 ; Summer - 53-68% ± 2 ; Winter - 44-64% ± 2.

During three successive years (2011-14) surveyed Kharif crop season i.e., July to October and Rabi crop season i.e., November to June in rice cropping seasons studied the physicochemical characters in different rice growing localities showed (**Table 1**). The character of soil color mostly grayish to blackish. Types of soil was sandy to loamy and clayey. Average temperature was in summer: 26 – 41 ± 2^{0}C but maximum were in May 41 ± 2^{0}C. But minimum were in December to January. Its range were 8 to 26 ± 2^{0}C. Average Humidity percentage range were July to September which were 67-89 ± 5%. In Winter it's were 44-64 ± 2%**.** Range of available Phosphorus (ppm) were 2.93 to 6.92 ± 0.5. The range of pH was from 5.10 to 8.53 ± 0.50 in November to July i.e., in Rabi crop season. The range of pH was minimum in October to June i.e., 5.10 ± 0.5 and maximum were in July to October i.e., 8.53 ± 0.50. The pH range were maximum in July to September i.e., 6.5 to 8.53 ± 1 and maximum in September i.e., 8.53 ± 1. In Haringhata, the minimum i.e., acidic. Were in July i.e., 6.5 at Kalyani in kharif season and moderate at Jaguliya. The range of conductivity (E.C. in dS/m) were <1 ± 0.2; range of organic carbon were from 0.52 – 1.04 ± 2%. Range of organic carbon percentage were minimum at Kalyani and maximum at Haringhata and moderate at Jaguliya. The range of total Nitrogen percentage were 0.15 to 0.92 ± 0.2 which were minimum at Kalyani i.e. 0.15 ± 0.20 and maximum range at Haringhata i.e., 0.92 ± 0.2 and moderate at Jaguliya.

Table 1. Physicochemical range of Nadia district.

Parameter	Data Range
pH range	5.10±0.50 to 8.53±0.50
Conductivity (E.C. in dS/m)	<1±0.50
Range of Organic carbon %	0.52 ± 0.20 to 1.04 ± 0.20
Range of Total Nitrogen %	0.15 ±0.20 to 0.92 ±0.50
Range of Available Phosphorus (ppm)	2.93±0.50 to 6.92±0.50

The pH range were maximum in September i.e., 8.64 ± 1 at Haringhata and the minimum were in July i.e., 6.9 at Kalyani. It showed maximum alkalinity in kharif season and followed Jaguliya and next Kalyani. The range of Conductivity (E.C. in dS/m) were <1±0.2; in all three blocks more or less same. Range of organic carbon percentage were from 0.52 – 1.04 ± 2. Range of organic carbon percentage were minimum at Kalyani and maximum at Haringhata and moderate at Jaguliya. The total nitrogen percentage were 0.15 to 0.92 ± 0.2. The percentage of nitrogen range were minimum at Kalyani i.e., 0.15 to 0.73 ± 0.20 and maximum Range at Haringhata i.e., 0.84 ± 0.2 to 0.92 ± 0.2 and moderate at Jaguliya **(Table 2).**

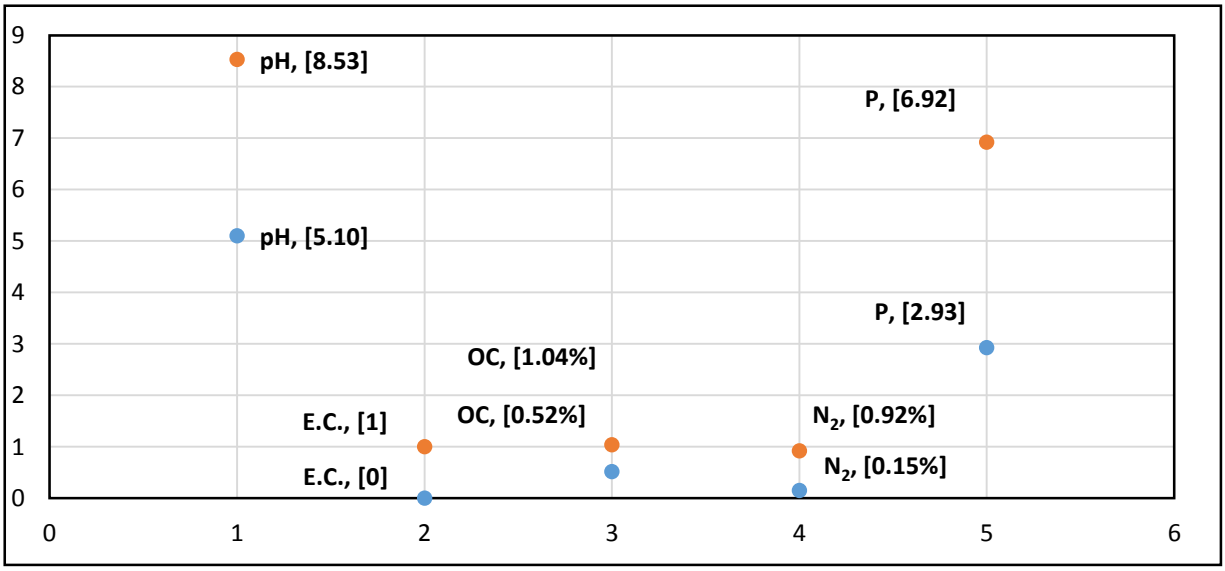

Figure 1: Physicochemical range of different parameters.

Table 2. Physicochemical status of 3 sites i.e., Haringhata, Kalyani and Jaguliya of Nadia district.

Collection Site	Year	OC (%)	pH range		Total Nitrogen (%)	Soil Texture	Range of Phosphorus (ppm)
			Kharif (July to Octo.)	Rabi (Nov. to June)			
Haringhata	2011	1.04±0.20	7.5±0.50	5.6±0.50	0.85±0.20	C & S	6.92±0.50
	2012	0.89±0.20	8.53±0.50	6.1±0.50	0.84±0.20	S & L	4.78±0.50
	2013	0.92±0.20	8.51±0.50	7.3±0.50	0.92±0.20	L & S	5.63±0.50
Kalyani	2011	0.86±0.20	7.27±0.50	6.52±0.50	0.15±0.20	S & L	5.60±0.50
	2012	0.52±0.20	6.5±0.50	5.10±0.50	0.73±0.20	C & S	4.5±0.50
	2013	0.82±0.20	7.8±0.50	6.38±0.50	0.62±0.20	C & L	2.93±0.50
Jaguliya	2011	0.98±0.20	7.88±0.50	6.29±0.50	0.58±0.20	Loam	4.9±0.50
	2012	0.92±0.20	7.90±0.50	6.19±0.50	0.72±0.20	C & S	5.48±0.50
	2013	0.81±0.20	8.48±0.50	6.22±0.50	0.63±0.20	Loam	5.61±0.50
C & S: Clay & Sandy; **S & L**: Sandy & Loam; **L & S** : Loam & Sandy; **C & L**: Clay &Loam							

Table 3. Habitat wise distribution pattern of cyanobacteria genera in different blocks of Howrah district:

Nature of the fields/Habitat	No. of times encountered	Occurrence Percentage
Up land	176	19.30%
Wet land	164	17.90%
Low land	155	16.20%
Soil Surface	282	38.40%
Usar land	94	8.20%
TOTAL	**871**	**100%**

Table 4. Cyanobacterial succession during irrigated rice cropping seasons from 2011 to 2014.

Name of the common Cyanobacteria	Cyanobacterial succession during rice cropping 3 different seasons					
	2011-2012		2012-2013		2013-2014	
	Kharif (July to Octo.)	Rabi (Nov. to June)	Kharif (July to Octo.)	Rabi (Nov. to June)	Kharif (July to Octo.)	Rabi (Nov. to June)
Chroococcales						
Merismopedia duplex	++	+	+	+	+	-
Gloeothece repestris	+	-	+	+	+	+
Microcystis flos-aquae	+	++	+	+	-	+
Chroococcus cyanosarcina	+	+	+	+	-	+
Gloeocapsa rupstris	-	+	-	+	-	+
Gloeocapsa biformis	++	+	-	+	-	+
Chroococcus cyanosarcina	-	+	+	++	-	+
Oscillatoriales						
Phormidium foveolarum	++	+	+	-	+	+
Phormidium molle	+	-	+	+	++	+
Phormidium tenue	++	+	++	-	+	+
Oscillatoria obscura	++	-	+	+	++	+
Oscillatoria formosa	+	+	++	-	+	+
Oscillatoria. animalis	+	+	++	+	++	+
Microcoleus sp.	+	+	+	+	+	+
Nostocales						
Nostoc paludosum	+	-	+	-	++	+
Nostoc linckia	++	-	+	+	-	+
Nostoc calcicola	-	+	+	-	+	-
Scytonema hofmani	+	++	+	+	+	-
Scytonema simplex	+	-	+	-	+	+
Captylonemopsis sp.	-	-	+	-	+	-
Anabaena fertilissima	++	+	+	+	++	+
Anabaena oryzae	+	+	+	-	+	+
Anabaena ambigua	++	+	+	+	+	-
Aulosira fertililissima	++	-	+	-	++	+
Calothrix javanica	++	-	++	+	+	-
Microchaete sp.	+	-	+	-	+	+
Tolypothrix byssoide	+	-	-	+	-	+
Tolypothrix tenuis	-	+	+	-	+	+
Microchaete tenera	+	-	+	-	-	+
Microchaete uberrima	+	-	+	-	+	-
Tolypothrix byssoide	+	-	-	+	-	+
Cylindrospermum indicum	++	-	+	-	+	+
Cylindrospermum majus	++	+	+	+	+	-
Cylindrospermum musicola	+	+	+	-	+	+
Gloeotrichia rupestis	++	+	+	-	+	-
Gloeotrichia ghosei	+	-	+	+	+	+
Stigonematales						
Westiellopsis prolifica	+	+	-	+	++	-

*** ++=More; += Minimum Present; - = Rare:**

Cyanobacteria have been found not only to grow in highly alkali soils but also improve the physicochemical properties of soils enriching them with carbon, nitrogen and available phosphorus Kaushik, B. D.(1994). Among the unicellular group, planktonic forms were fast growing and had less generation time. They were more sensitive due to environmental changes i.e., temperature, nutrients, pH level and water level. Rice fields of surveyed localities particularly showed abundant occurrence of Nostocacean flora by Y.P. Singh, Rama Kant, N. C. Halder and G.L. Tiwari 2010; Similar variations from rice fields had also been reported by other workers Deka and Bordoloi, 1992; Saikia and Bordoloi, 1994; Tiwari, .G. L., Singh, Y.P., Rama K., Halder, N. C. (2008), Singh, B.V., and Singh, (2001). The incidence of total number of samples were recorded from different localities of 3 different sites in Nadia district with reference to genera as well as Eco-physiochemical studies which are presented in Table 1-5. Most of the paddy fields under investigations, they do not have submerged condition and a large number of BGA grow on soil surface. They form mixed and irregular patches of different colours and consistency. Among three sites, Haringhata exhibited the maximum number of cyanobacteria and the moderate at Kalyani and minimum at the Jaguliya due to presents of different physicochemical parameters were suitable for growth of BGA accordingly. The paddy field ecosystem consists of diverse habitats for microorganism. The abundance of cyanobacteria in paddy fields was first observed by Fritsch (1907).

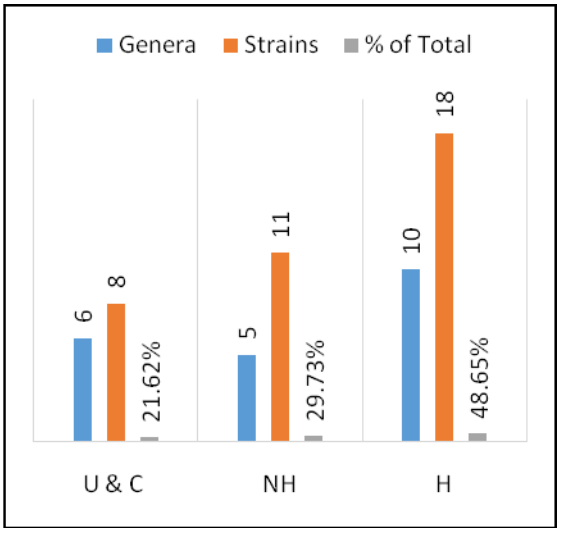

Figure 2: Habitat wise distribution pattern of Cyanobacteria genera (U & C = Unicellular and Colonial Cyanobacteria; NH = Non-Heterocystous Cyanobacteria; H= Heterocystous Cyanobacteria).

Table 5. Occurrence of different groups of Cyanobacteria in rice fields of Nadia district.

Cyanobacteria	Genera	Strains	Percentage of Total
Unicellular and Colonial	6	08	21.62%
Non-Heterocystous filamentous	5	11	29.73%
Heterocystous	10	18	48.65%
TOTAL	21	37	100%

Exorbitant use of agrochemicals to increase soil fertility and crop productivity are reported to substantially reduce or eliminate cyanobacterial flora from paddy fields (Stewart et al., 1972; Susheela and Goyal, 1995; Nike et al., 1997). During the study altogether 871 times representation of 37 species of Nitrogen fixing Cyanobacteria samples and it belonging to 21 genera and four orders i.e., Chroococcales, Oscillatoriales, Nostocales, and Stigonematale were isolated from various rice growing localities. Three major Cyanobacterial groups (**Table 5**):

i. Unicellular and colonial were minimum i.e., 21.62 % and its 6 genera and 8 species.

ii. Non-heterocystous filamentous Cyanobacteria were slight more i.e., 29.73% and its 5 genera and 11 species

iii. Heterocystous filamentous cyanobacteria were maximum i.e., 48.65%, its

10 genera and 18 species were occurrence in most of all low and upland rice growing areas. Habitat and encountered wise Distribution Pattern of Cyanobacteria genera (Table-3) were maximum in soil surface areas, there were 38.40% and the followed in upland i.e., 19.30% then followed wetland i.e., 17.90% then lowland i.e., 16.20% and minimum in usar land i.e., 8.20% successively depending upon the pH, EC, Temperature, humidity, total nitrogen, organic carbon, soil N, P, K and soil texture. Cyanobacteria benefits in rice plants by producing growth promoting substances followed by increasing the availability of phosphorus by excretion of organic acids was also exploited in the prevention of soil erosion process (Kumar and Rao, 2012). BGA inoculation popularly known as "Algalization" helps to provide an environmentally safe agro-ecosystem contributing to economic viability in paddy cultivation, reducing cost and energy inputs (Pabbi, 2008).The favorable balance of soil nitrogen of rice fields wherein rice can be grown on the same land even groups of Cyanobacteria i.e. the order Chroococcales and minimum the order Oscillatoroales and increasing the pH and water label up to 14cm and decrease light intensity in Kharif crop season developed mostly filamentous group of Cyanobacteria i.e., Oscillatoroales, Nostocaleand Stigonematales. Among three sites, Haringhata exhibited the maximum number of cyanobacteria and the moderate at Jaguliya blocks and minimum at the Kalyani blocks due to presence of different physicochemical parameters for growth of BGA i.e., pH, temperature, water level, nitrogen and carbon percentage accordingly. The common forms are *Nostoc calcicola, Anabaena fertilissima, Oscillatoria formosa, Aphanocapsa sp., Scytonema simplex and Cylindrospermum indicum, Microchaete sp.,Phormidium molle, Gloetorichia rupestris* and Lyngbya *sp.* It is very clear that the without any addition of fertilizers and without any reduction in yield, confirms to the significance of cyanobacterial nitrogen fixation (Venkatraman, 1972; Nayak et al., 2001; Song et al., 2005) .These habitats are micro environment physico-chemically different to each other and could exhibits biologically distinct properties. Such heterogeneity of the habitat should influence the structure and diversity of microbial communities in the paddy field ecosystem as a whole and may support various microbiological process occurring in paddy fields which are agronomic ally and bio geochemically important (Kimura, 2000; Kirk, 2004).

Conclusion

Present investigations revealed that the pH range has a great role for the growth and succession of cyanobacteria. Low range of pH and more light intensity in Rabi crop season developed mostly the strains of unicellular

growth of free floating separate and distinct colonies of BGA eg., *Aulosira fertilissima, Anabaena oryzae, Aphanothece sp., Gloeotrichia rupestris* and *Microchaete tenera* largely dependent on water level and generally they do not grow attached on soil surface. The Unicellular and colonial were forms were more in March to June i.e., beginning of the crop and Non-heterocystous and Non-heterocystous forms developed middle to end of the crop seasons.

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