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Cyanobacterial diversity and physicochemical study of different blocks of Howrah district in West Bengal, India

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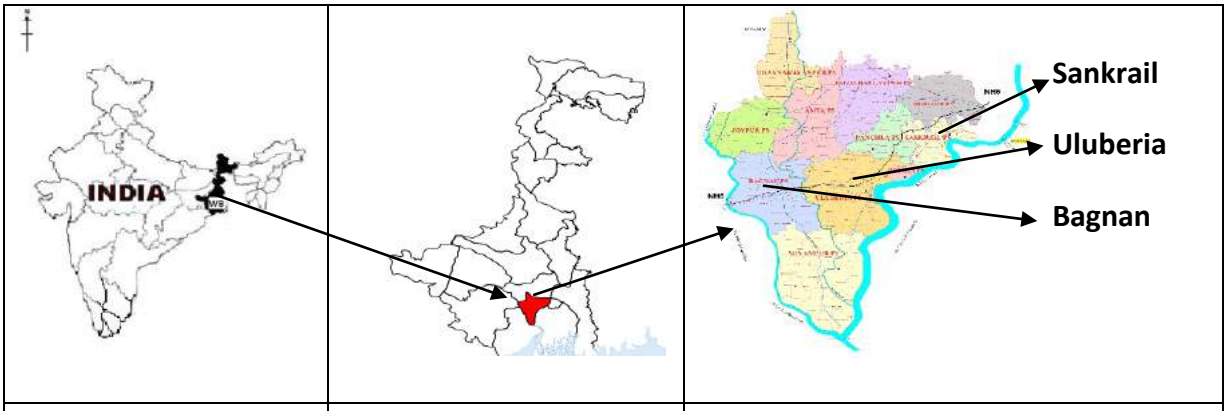
Abstract

Present research paper deals with continued three successive years (2011 - 2014) studied for the first time the occurrence of Cyanobacteria in rice growing fields of Howrah district in West Bengal. Altogether 847 times representation of 38 species of Nitrogen fixing Cyanobacteria samples belonging to 22 genera and four orders i.e., Chroococcales, Oscillatoriales, Nostocales and Stigonematales were isolated from various rice growing localities. The physicochemical 11 parameters i.e., pH, EC, Temperature, Humidity, Total Nitrogen, Organic Carbon, C/N ratio, soil N, P, K and soil texture were chosen for this study. Present study revealed that comparatively less pH and lesser number of Cyanobacteria were growing in Rabi i.e., November to June in comparison to Kharif crop season i.e., July to October. The increasing range of soil pH was certainly the important factor for the development of BGA.

Keywords: Cyanobacteria, distribution pattern, ecology, physicochemical.

Introduction

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 wide spread distribution and tolerance to environmental stress (Tandeau de Marsac and Howard, 1993). It can play an important role in maintenance and build-up of soil fertility, consequently increase rice growth and yield as a natural bio fertilizer. Exorbitant use of agrochemicals to increase soil fertility and crop productivity are reported to substantially reduce or eliminate cyanobaterial flora from paddy fields (Stewart et al., 1972; Susheela and Goyal 1995; Nike et al., 1997). Cyanobactaria 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 cyanobaterial flora



West Bengal in India	Howrah in West Bengal	Three blocks in Howrah Districts

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 cyanobacteria biodiversity of three different major Blocks i.e., Uluberia-II, Sankrail and Bagnan and their adjoining areas of Howrah district in West Bengal, India.

Materials and Methods

1. Isolation of the culture

Cyanobacteria samples along with soil and water were collected from major rice growing three Blocks i.e., Uluberia-II, Sankrail and Bagnan and their adjoining areas of Howrah 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 150 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) and one ml of this suspension was spread over the same medium gelled with 1% (w/v) agar-agar poured in the petridishes. The samples were first studied under stereomicroscope for their general morphological features like nature of colony, colour and general appearance of thallus in details.

2. 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 media, 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 30^0C ± 2 and 4000-5000 Lux light intensity under 14/10 LD cycle.

3. Maintain of Cyanobacterial strains:

All the isolated strains of Cyanobacteria are being maintained in culture collection in the Department of Botany, Uluberia College (University of Calcutta), Howrah, West Bengal, India.

4. Physico-chemical 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.

5. 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, akinete and branches.

Results and Discussion

Meteorological and Effect of Physico-chemical parameters

Howrah 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$48' N to 22^0 12' N Lat. and $88^0$23' E – $87^0$50' E Long., and covers area about 1467 sq. kms in West Bengal. 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 localities showed (Table-1). The character of Soil color mostly Grayish to blackish. Types of Soil are Sandy to loamy and clayey. Average temperature was in summer: 27 – 42^0C ± 2^0C but maximum were in May 42^0C ±2^0C. But minimum were in December to January and its range were 8.5 to9.5. ± 1^0C; Average Humidity (%) range were July to September were 68 -88±5 %, In Winter: 45-62 %±5.

Range of Available Phosphorus (ppm) were 2.63 to 6.69±0.5; Range of C/N were7.10 to 9.44±1. The pH range were from 5.38 to 8.64 ± 0.50 in November to July i.e., in Rabi crop season. The pH range was minimum in October to June i.e., 5.38±0.5 and maximum in July to October i.e., 8.64±0.50. The pH range were maximum in July to September i.e., 6.9 to 8.94 ± 1. The pH range were maximum September i.e., 8.64±1 in Uluberia block II and the minimum were in July i.e., 6.9. Uluberia II block Showed maximum alkalinity in kharif season and followed Bagnan block and next Sankrail Block. The range of Conductivity (E.C. in dS/m) were <1 ± 0.2; Range of Organic carbon % were from 0.55 - 0.99 ± 2. Range of Organic carbon % were minimum in Sankrail block and maximum in Block Uluberia II and moderate in Bagnan block. The Total Nitrogen % were0.17 to 0.89±0.2 Range were minimum in Sankrail block i.e., 0.48±0.20 and maximum in Range in Block Uluberia II i.e., 0.89 ± 0.2 and moderate in Bagnan block.

Diversity of Cyanobacteria

The incidence of total number of samples were recorded from different localities of 3 different blocks in Howrah district with reference to genera as well as Eco-physiochemical studies which are presented in Table 1-6. 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 Blocks Uluberia-II exhibited the maximum number of cyanobacteria and the moderate at Bagnan blocks and minimum at the Sankrail blocks due to presents of different physio-chemical parameters were suitable for growth of BGA accordingly. The paddy field ecosystem consists of diverse

Table 1. Detailed survey have been made and collected data are given below

Geographical Location		**Character Of Soil**		**Weather Record**		
Total area of Howrah district: 1,467 km^2		**Soil Color**	**Grayish to blackish**	**Average Rainfall**	**June to September**	**278 mm ±5**
Latitude	22^{0}48'N-22^0 12' N	Soil Type	Sandy to loamy and clay	Average Temperature	Summer	(27±2 to 42±1) ^{0}C
					Winter	(8.5- 27±1) ^{0}C
Longitude	88^{0}23'E–87^{0}50'E	Average Range Humidity (%)				
		July to September		Summer	Winter	
		68 to 88% ± 5		52-67 % ± 5	45-62 % ± 5	

habitats for microorganism. The abundance of cyanobacteria in paddy fields was first observed by Fritsch, 1907.

Table 2. Physicochemical range of Howrah district.

Parameter	Data Range
pH range	5.38±0.50 to 8.64±0.50
Conductivity (E.C. in dS/m)	<1±0.50
Range of Organic carbon %	0.55 ± 0.20 to 0.99 ± 0.20
Range of Total Nitrogen %	0.17 ±0.20 to 0.89 ±0.50
Range of Available Phosphorus(ppm)	2.63±0.50 to 6.69±0.50
Range of C/N	7.58±0.50 to 9.44±0.50

During the study altogether 897 times representation of 38 species of Nitrogen fixing Cyanobacteria samples and it belonging to 22 genera and four orders i.e., Chroococcales, Oscillatoriales, Nostocales, and Stigonematales were isolated from various rice growing localities. Three major Cyanobacteial groups (Table-5): i. Unicellular and colonial were minimum i.e., 22.5% and its 7 genera and 8 species. ii. Non-Heterocystous filamentous Cyanobacteria were slight more i.e., 25.0% and its 4 genera and 8 species iii. Heterocystous filamentous Cyanobacteria were maximum i.e., 52.5%, its 11 genera and 22 species were occurrence in most of all low and upland rice growing areas. Habitat and encountered wise Distribution Pattern of Cyanobacteria genera (Table-4) were maximum in soil surface areas, there were 38.55% and the followed in lowland i.e., 18.40% then upland i.e., 18.20% then wetland i.e., 17.60% and minimum in Usarland i.e., 7.25% successively depending upon the. pH, EC, Temperature, Humidity, Total Nitrogen, Organic Carbon, C/N ratio, soil N, P, K and soil texture.

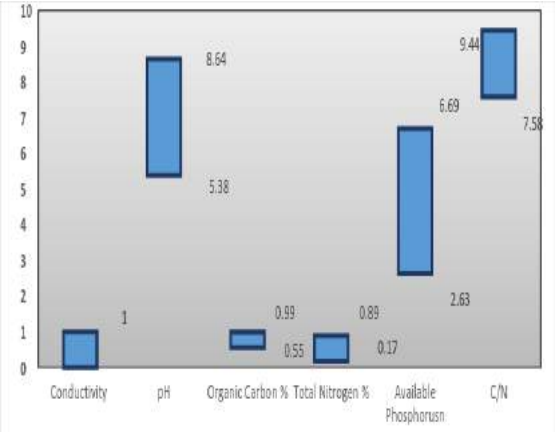


Figure 1. Physicochemical range of different parameter.

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).

Table 3. Physicochemical status of 3 Blocks i.e., **Uluberia-II, Sankrail and Bagnan of Howrah district.**

BLOCKS	YEAR	OC (%)	pH range		Total Nitrogen (%)	Soil Texture	Range of C/N	Range of Phosphorus (ppm)
			Kharif (July to Octo.)	Rabi (Nov. to June)				
Uluberia-II	2012	0.96±0.20	6.9±0.50	5.8±0.50	0.89±0.20	C & L	8.7±0.50	5.60±0.50
	2013	0.79±0.20	8.64±0.50	8.1±0.50	0.81±0.20	S & L	8.85±0.50	4.98±0.50
	2014	0.64±0.20	8.1±0.50	7.3±0.50	0.83±0.20	L	9.44±0.50	6.69±0.50
Sankrail	2012	0.55±0.20	7.64±0.50	6.52±0.50	0.48±0.20	S & L	7.1±0.50	4.69±0.50
	2013	0.65±0.20	7.38±0.50	6.55±0.50	0.63±0.20	C	8.3±0.50	3.5±0.50
	2014	0.47±0.20	7.8±0.50	7.38±0.50	0.57±0.20	C & L	7.44±0.50	2.63±0.50
Bagnan	2012	0.78±0.20	7.38±0.50	6.38±0.50	0.50±0.20	L	8.1±0.50	2.9±0.50
	2013	0.61±0.20	7.80±0.50	5.49±0.50	0.89±0.20	S & L	8.60±0.50	6.4±0.50
	2014	0.68±0.20	7.98±0.50	6.4±0.50	0.69±0.20	C & L	7.98±0.50	4.63±0.50

*** L = Loam ; **C = Clay ; *** S & L = Sand & Clay & **** C & L = Clay & Loam**

Table 4. Habitat wise distribution pattern of cyanobacteria genera in different blocks of Howrah district:

Nature of the fields/Habitat	No. of times encountered	% occurrence
Up land	163	18.20%
Wet land	158	17.60%
Low land	165	18.40%
Soil Surface	346	38.55%
Usar land	65	7.25%
TOTAL	**897**	**100%**

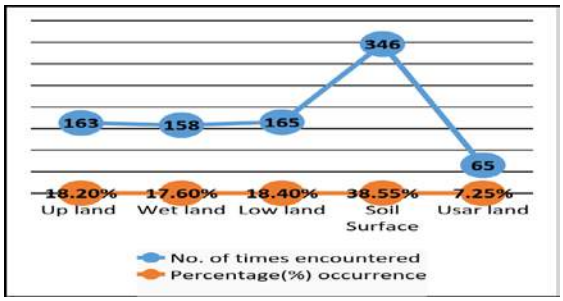


Figure 2. Habitat wise distribution pattern of Cyanobacteria genera.

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).

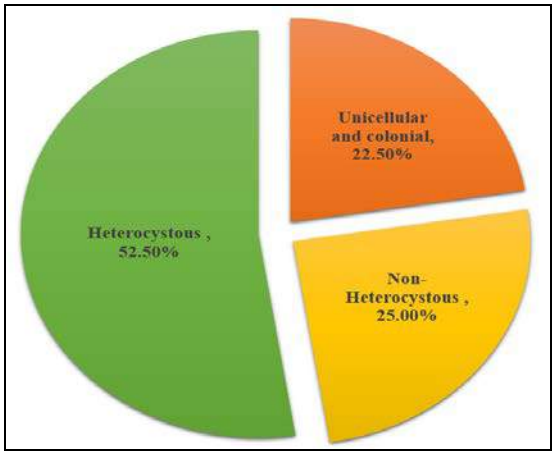


Figure 3. Occurrence (%) of different groups of Cyanobacteria.

Table 5. Occurrence of different groups of Cyanobacteria in rice fields of Howrah District.

Cyanobacteria	Strains	% of Total
Unicellular and colonial Cyanobacteria	9	22.5%
Non-Heterocystous filamentous Cyanobacteria	10	25.0%
Heterocystous Cyanobacteria	21	52.5%
TOTAL	**40**	**100%**

Table 6. Cyanobacterial succession during irrigated rice cropping seasons from 2011 to 2014.

Group	Orders	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)
A	Chroococcales	*Aphanothece sp.*	P	PP	R	P	R	PP
		Chroococcus cyanosarcina	R	P	P	PP	R	P
		Gloeocapsa rupstris	R	P	R	P	R	P
		Gloeocapsa biformis,	P	PP	R	P	R	PP
		Merismopedia duplex	R	P	R	P	P	P
		Gloeothece repestris,	P	P	R	PP	R	P
		Microcystis flos-aquae	P	PP	R	P	R	P
		Aphanocapsa sp.	R	P	R	P	R	P
B	Oscillatoriales	*Lyngbya rgie*	PP	P	PP	P	PP	P
		Microcoleus sp.	P	P	P	P	P	P
		Oscillatoria. animalis,	P	P	PP	P	PP	P
		Oscillatoria Formosa	P	P	PP	R	P	P
		Oscillatoria obscura	PP	R	P	P	PP	P
		Phormidium tenue	PP	P	P	R	P	R
		Phormidium molle	P	R	PP	P	PP	P
		Phormidium foveolarum	P	R	P	R	P	P
C	Nostocales	*Anabaena fertilissima*	PP	R	P	P	PP	P
		Anabaena oryzae	PP	P	P	R	P	P
		Anabaena ambigua	PP	P	PP	P	P	R
		Aulosira fertililissima	PP	R	PP	R	PP	P
		Calothrix javanica,	PP	R	PP	P	P	R
		Calothrix marchica	P	P	P	R	P	P
		Cylindrospermum indicum	PP	R	P	R	P	P
		Cylindrospermum majus	PP	P	P	P	P	R
		Cylindrospermum musicola	P	R	P	R	P	P
		Gloeotrichia rupestis	PP	P	P	R	PP	R
		Gloeotrichia ghosei	P	R	P	P	R	P
		Microchaete sp.	P	R	P	R	P	P
		Nostoc paludosum	PP	R	PP	R	PP	P
		Nostoc linckia	PP	R	P	P	R	P
		Nostoc calcicola	R	P	P	R	P	R
		Scytonema hofmani	R	P	P	P	P	R
		Scytonema simplex	P	R	P	R	P	P
		Captylonemopsis sp.	R	R	P	R	P	R
		Tolypothrix byssoide	P	R	R	P	R	P
		Tolypothrix tenuis	R	P	P	R	R	P
		Microchaete tenera	P	R	P	R	R	P
		Microchaete uberrima	P	R	P	R	R	R
	Stigonematal-es	*Westiellopsis prolifica*	R	P	R	P	P	R
		Hapalosiphon welwitschii	P	P	R	P	R	P

***A = Unicellular and colonial; B = Non-Heterocystous Cyanobacteria: C = Heterocystous Cyanobacteria**

**** PP=More; P= Minimum Present; R= Rare**

The favourable balance of soil nitrogen of rice fields wherein rice can be grown on the same land even 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, physicochemically 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). 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, 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 Singh, Y.P., Kant, R., Halder, N. C., and Tiwari, G.L., 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., Kant, R. and Halder, N. C., 2008; Singh and Singh, 2001.

Conclusion

The common genera *are Nostoc linckia, Anabaena ambigua, Oscillatoria formosa, Aphanocapsa, Scytonema simplex, Cylindrospermum majus, Microchaete tenera, Phormidium tenue, Gloetorichia rupestis and Lyngbya rgie.* It is very clear that the growth of free floating separate and distinct colonies of BGA eg. *Aulosira fertilissima, Anabaena ambigua, Aphanothece, Gloeotrichia rupestis and Microchaete tenera* largely dependent on water level and generally they do not grow attached on soil surface. 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 develops mostly the strains of unicellular groups of Cyanobacteria i.e., the order Chroococcales and minimum the order Oscillatoroales and increasing the pH ,and water label up to 12cm and decrease light intensity in Kharif crop season developed mostly filamentous group of Cyanobacteria i.e., Oscillatoroales, Nostocales and Stigonematales. Among three Blocks, Uluberia-II exhibited the maximum number of cyanobacteria and the moderate at Bagnan blocks and minimum at the Sankrail blocks due to presence of different physio-chemical parameters for growth of BGA accordingly.

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