Bangladesh J. Bot. **37**(2): 183-188, 2008 (December)

QUANTIFICATION AND NITROGEN FIXATION OF CYANOBACTERIA IN RICE FIELD SOILS OF BANGLADESH

Z.N. Tahmida Begum*, R. Mandal[1] and Farzana Binta Amin

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Cyanobacteria, Quantification, N_2-fixation, Rice field soil

Abstract

Quantification of cyanobacterial population and nitrogen fixation of 18 heterocystous cyanobacteria isolated from rice fields soils of 11 districts of Bangladesh have been studied. The taxa recorded were, namely *Nostoc linckia, N. carneum, N. ellipsosporum, N. piscinale, Anabaena oryzae, Scytonema mirabile, Calothrix marchica* and *Hapalosiphon welwetschii.* The number of cyanobacterial population varied significantly ranging from 14.6×10^4 to 141.0×10^4/g soil. N_2-fixation of *Nostoc linckia* isolated from rice fields of Dhaka and Brahmanbaria ranged from 1.84 to 8.13 mg N 50/ml. However, the potentiality of other species was more or less similar. Single correlation study suggests that quantitative variation in cyanobacterial population was significantly related positively to soil reaction, available P and available S and negatively to total N content of soil. However, multiple correlations reflects that variation of cyanobacterial population was due to cumulative contribution of all the variables.

Introduction

In the process of biological nitrogen fixation in rice field ecosystem the inherent nitrogen fixing capacity of indegenous cyanobacteria is one of the most important factors (Kaushik 2002). Since the report of the capability of fixing atmospheric nitrogen to soil by De (1939), in Bangladesh not much initiative was taken before 1983 (Begum 1983) in this respect. So far the distribution of cyanobacteria in the rice fields of Bangladesh have been made by Aziz *et al.* (1992), Begum (1983) and Khan and Venkataraman (1991) and impact of algalization on the growth and yield of rice in pot culture (Begum and Islam 1982, Mannan *et al.* 1986 and Begum *et al.* 1990) and in fields (Bhuiya *et al.* 1984) have been investigated without prior testing their potential nitrogen fixing capacity. However, Rother *et al.* (1988), Begum and Mandal (1997) and recently Gafur and Parvin (2008) have reported about the nitrogen fixing potential of some isolated cyanobacteria. Thus, in the present investigation, an attempt has been made to evaluate the quantitative status and nitrogen fixing potential of cyanobacteria isolated from rice fields of Bangladesh encompassing wide range of soil properties.

Materials and Methods

Soil samples (0-5 cm, 0-15 cm) were collected from 44 rice fields covering 11 districts of Bangladesh. Each sample was a composite sample of 5 subsamples. Nitrogen free Fogg's medium was used for isolation and consecutive experiments. Cyanobacterial taxa were identified by standard texts following their growth in the flasks and plates. Quantative enumeration of cyanobacteria in the different soil samples was determined by MPN method. Determination of pH, organic carbon (Walkley and Black 1934), available phosphorus (Williams and Stewart 1941) available sulphur (Fox *et al.* 1964, Sakai 1978) and total N (Jackson 1962) of the soils and nitrogen fixed by the cyanobacteria (Jackson 1962) were measured by standard analytical techniques. The rice field soil environment is given in Table 1. One ml of uniform suspension of 4-day-old culture of each cyanobacterial taxa was separately used to inoculate in 150 ml flasks containing 50 ml of sterilized N-free Fogg's medium, in triplicate. Thirty-day-old cultures of cyanobacteria were used for the determination of nitrogen fixing capacity.

*Corresponding author.E-mail: <botany@univdhaka.edu> [1]Department of Soil, Water and Environment, University of Dhaka, Dhaka-1000, Bangladesh.

Table1. Chemical properties of rice field soils of different districts of Bangladesh.

Name of districts	Field No.	Cropping pattern	pH	Org. C	Total N	P	S
				Per cent		µg/g soil	
Dhaka division							
Dhaka	1	Rice	5.4	1.31	0.18	2.08	18.50
	2	,,	5.2	1.79	0.24	2.08	21.40
	3	,,	6.9	1.79	0.24	2.05	11.50
	4	,,	5.4	1.58	0.22	2.08	14.30
Faridpur	1	,,	7.6	0.96	0.15	3.00	43.75
	2	,,	7.5	0.81	0.14	3.50	46.80
	3	,,	7.4	0.51	0.08	2.40	38.50
	4	,,	7.5	0.57	0.08	4.50	35.20
Gazipur	1	,,	7.0	0.75	0.05	4.68	37.50
	2	,,	7.1	0.58	0.04	12.48	40.60
	3	,,	7.2	0.56	0.03	15.20	30.00
	4	,,	6.5	0.42	0.03	16.00	35.50
Kishoregonj	1	,,	5.3	0.26	0.04	4.20	21.88
	2	,,	5.5	1.17	0.17	3.20	7.81
	3	,,	5.3	1.51	0.21	2.20	7.00
	4	,,	6.5	1.56	0.22	3.70	7.50
Manikgonj	1	,,	5.5	0.83	0.15	2.28	28.20
	2	,,	5.5	0.78	0.16	4.50	37.50
	3	,,	5.8	0.76	0.15	3.20	25.50
	4	,,	5.9	0.80	0.12	2.00	35.00
Munshigonj	1	Rice-Rabi crops	5.4	1.21	0.16	12.08	15.60
	2	,,	5.4	1.43	0.21	17.28	10.30
	3	,,	5.4	1.50	0.22	17.31	11.60
	4	,,	5.9	1.60	0.23	7.00	10.00
Narayangonj	1	Rice	7.0	2.44	0.22	5.41	65.60
	2	,,	6.1	1.01	0.14	4.40	18.10
	3	,,	5.9	0.96	0.15	3.20	20.00
	4	,,	6.2	1.02	0.11	2.20	25.00
Narshingdi	1	Rice-Rabi crops	7.1	0.85	0.06	7.00	13.75
	2	,,	7.2	0.88	0.07	14.68	12.50
	3	,,	7.3	0.72	0.05	12.28	13.00
	4	,,	7.2	0.86	0.07	6.68	12.00
Chittagong division							
Brahmanbaria	1	,,	6.8	0.44	0.05	28.50	62.50
	2	,,	6.9	1.11	0.06	27.30	19.30
	3	,,	7.5	1.04	0.07	12.42	20.25
	4	,,	7.2	1.06	0.18	16.50	25.00
Comilla	1	Rice	5.8	0.62	0.08	2.06	12.13
	2	,,	6.1	0.33	0.04	2.02	9.52
	3	,,	5.5	0.34	0.03	2.00	9.00
	4	,,	5.6	0.60	0.05	2.10	12.00
Khulna division							
Khulna	1	,,	7.3	0.95	0.14	5.00	21.80
	2	,,	6.5	1.08	0.10	4.00	35.00
	3	,,	7.5	1.41	0.21	4.20	25.50
	4	,,	7.1	1.50	0.12	4.10	46.80
Overall	Maximum		7.6	2.44	0.24	28.50	65.60
	Minimum		5.2	0.26	0.03	2.00	7.00
	Mean		6.4	1.00	0.12	7.16	24.32

Results and Discussion

Quantitative assessment of cyanobacteria: A significant variation in cyanobacterial population was observed among the fields of each district and also among the districts (Table 2). The number of cyanobacteria varied significantly ranging from 14.6 to 141.0 × 10^4/g soil. Variation in heterocystous cyanobacterial population in rice field soils of India was also recorded by Roger *et al.* (1986).

Table 2. Quantitative distribution of indigenous cyanobacteria in rice field soils of 11 districts of Bangladesh.

Sl. No.	Districts	No. cyanobacteria × 10^4/g soil				Mean	LSD P=0.05
		Field-I	Field-II	Field-III	Field-IV		
1	Brahmanbaria	125.0	104.0	81.8	141.0	109.62	5.65
2	Comilla	21·2	14.6	14.7	12.8	15.82	NS
3	Dhaka	14·6	21.0	16.6	21.2	18.35	1.81
4	Faridpur	41.6	42.5	38.1	43.5	41.42	NS
5	Gazipur	48·3	58.8	70.0	81.8	64.72	4.12
6	Khulna	31.6	21.0	27.0	16.6	24.05	2.85
7	Kishoregonj	16·6	21.0	18.7	21.7	19.5	0.82
8	Manikgonj	14·6	18.6	16.6	14.7	16.12	1.55
9	Munshigonj	39·9	47.4	38.8	45.0	42.77	1.22
10	Narayangonj	42.5	21.2	21.0	23.0	26.92	NS
11	Narshingdi	47·4	69.2	56.3	70.0	60.72	3.45
	LSD P=0.05	2·01					

The highest number of indigenous cyanobacterial population in the rice field soils of Brahmanbaria might be due to neutral pH range (6.8 to 7.5) and higher amount of available phosphorus (12.42 to 28.50 μg/g soil) in comparison to soils of other districts (Table 1).Values represented in Comilla district are much higher than the values reported from the rice field soils in Thailand (Araragi and Tangcham 1979). The reason might be due to the favourable soil conditions for the growth of cyanobacteria prevailing in Bangladesh (Table 1).The recorded lower range of cyanobacteria is in good agreement with previous findings from Dhaka (Mandal *et al.* 1993), Khulna (Biswas 1993) and Comilla, Dhaka, Kishoregonj, Manikgonj and Naryangonj (Hossain 1992).

Table 3. Co-efficients of single (r) and multiple (R) correlation between cyanobacterial population and fertility variables.

Correlation		Soil fertility variables				
		pH	Organic C	Total N	Available P	Available S
Single (r)	Cyanobacterial population	0.5065 (P = 0.001)	– 0.0970 NS	– 0.2820 (P = 0.05)	0.8414 (P = 0.001)	0.2424 (P = 0.1)
Multiple (R)					0.8789 (P = 0.001)	

The quantitative variation in cyanobacterial population was found to be significant at 5% level among the soil samples collected from 11 districts of Bangladesh. The variation in cyanobacterial population is positively and significantly related to pH, available P and available S while negatively but significantly to total N contents of the soils (Table 3). However, multiple correlation suggests that the joint contribution of pH, organic C, total N, available P and available S is significantly responsible so far the variation in the abundance of cyanobacteria is concerned in the rice fields investigated.

Nitrogen fixing capacity of cyanobacteria: A wide variation in the ability of nitrogen fixing capacity is evident among 18 isolates of cyanobacteria (Table 4). Analytical data revealed that the range in amount of nitrogen fixed by *N. linckia* per 50/ml/30 days varied from 1.84 to 8.13 mg N 50/ml/30 days. However, in contrast, almost identical capacity in N_2-fixation by *Nostoc carneum* (3.25 to 3.27 mg N), *Nostoc ellipsosporum* (4.71 to 5.29 mg N), *Hapalosiphon welwitschii* (3.10 to 3.75 mg N), *Calothrix marchica* (2.28 to 2.68 mg N) and two isolates of *Scytonema mirabile* (4.34 to 4.87 mg N) has been observed. Previously Gafur and Parvin (2008) reported 3.38 mg N/g dry biomass at 30 days old culture solution of *Nostoc spongiaeformae*.

Table 4. Nitrogen fixation capacity of cyanobacterial isolates.

Sl. No.	Isolate No.	District	Cyanobacteria	N_2- fixation (mg N 50/ml/ 30 days)	Mean N_2- fixation (mg N 50/ml/30 days) of different taxa
1	Nl	Dhaka	*Nostoc linckia*	1.84	
2	Nl-4	Kishoregonj	*N. linckia*	4.09	
3	Nl-1	Comilla	*N. linckia*	4.86	4.84
4	Nl-3	Munshigonj	*N. linckia*	5.29	
5	Nl-2	Brahmanbaria	*N. linckia*	8.13	
6	Nc-1.	Brahmanbaria	*N.carneum*	3.25	
7	Nc-2	Faridpur	*N.carneum*	3.27	3.26
8	Ne-2	Narshingdi	*N.ellipsosporum*	4.71	
9	Ne-1	Khulna	*N.ellipsosporum*	5.29	5.00
10	Np-1	Munshigonj	*N. piscinale*	5.74	
11	A-1	Khulna	*Anabaena oryzae*	4.30	
12	Sc-3	Brahmanbaria	*Scytonema mirabile*	2.69	
13	Sc-2	Gazipur	*S. mirabile*	4.34	3.97
14	Sc-1	Narayangonj	*S. mirabile*	4.87	
15	Cm-1	Narayangonj	*Calothrix marchica*	2.28	
16	Cm-2	Manikgonj	*C. marchica*	2.68	2.48
17	H-2	Faridpur	*Hapalosiphon welwitschii*	3.10	
18	H-1	Munshigonj	*H. welwitschii*	3.75	3.43
Maximum				8.18	
Minimum				1.84	
Mean				4.13	

Observation on the variation in the amount of nitrogen fixation within the same species of a genus agreed favourably well with the results reported earlier by Hashem *et al.* (1994), Begum and Mandal (1997), Suseela and Goyal (1995) and Ahmed and Kalita (2002). In the present study, the amounts of nitrogen fixed by *Nostoc linckia* were 4.09 and 4.86 mg 50/ml/30 days isolated from the rice field soils of Kishoregonj and Comilla districts respectively (Table 4). These values are very close to the value (4.69 mg N 50/ml/30 days) reported for *N. linckia* isolated from rice fields of Moheshkhola and Norail districts (Begum and Mandal 1997).

On an average the efficiency of nitrogen fixing capacity (mg N 50/ml/30 days) of cyanobacterial genera irrespective of the species varied markedly and can be arranged as: *Nostoc piscinale* (5.74) > *N. ellipsosporum* (5.00) > *Nostoc linckia* (4.84) > *Anabaena oryzae* (4.30) > *Scytonema mirabile* (3.97) > *Hapalosiphon welwitschii* (3.43) > *Nostoc carneum* (3.26) > *Calothrix marchica* (2.48). However, the overall mean of N_2-fixing capacity was estimated to be 4.13 (mg N 50/ml/ 30 days) which can be considered good. This indicates the significance of cyanobacteria in rice fields and their contribution to nitrogen economy in soils of Bangladesh.

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(Manuscript received on 22 October, 2008; revised on 29 October, 2008)