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NITROGEN FIXING CAPACITY OF SOME CYANOBACTERIAL STRAINS FROM BANGLADESH

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Nitrogen fixing potential of 44 isolated heterocystous cyanobacteria belonging to 8 taxa namely *Nostoc* spp., *Anabaena variabilis, Aulosira aenigmatica, Scytonema mirabile, Calothrix* spp., *Hapalosiphon* spp., *Fischerella* spp. and *Stigonema* spp. was estimated. These strains were recovered from 37 soil samples collected from 26 districts of Bangladesh. Wide variations with a range from 0.378 mg N 50 ml^{-1} (in *Stigonema* sp.) to 5.41 mg N 50 ml^{-1} (in *Nostoc commune*) were observed in the nitrogen fixing ability of these isolates in liquid culture.

Keywords: Correlation coefficient; Cyanobacteria Fertility; Heterocystous; N_2 fixation; Rice soil.

Introduction

In most of the tropical and sub-humid tropical environment, cyanobacteria (blue-green algae) play an important role in improving the soil structure and fertility of the rice field soils and also in regulating the activities of soil microflora[1]. In addition to environmental conditions, nitrogen fixing capacity of the cyanobacteria is one of the most important factor for biological nitrogen fixation in rice field ecosystem[2,3]. In Bangladesh, the impact of algalization on the growth and yield of rice in pot culture[4-7] and fields[8] have been carried out to a limited extent without prior testing of their ability to fix nitrogen in the laboratory and also the extent of their viability as soil based inoculum. Mandal *et al.*[9] made some effort to assess the quantitative status of soil algae particularly the heterocystous, diazotropic cyanobacteria and the contribution of soil fertility factors in their variations in a wide range of rice soils. Report on the distributional pattern of 93 cyanobacteria in rice fields was made by Begum *et al.*[10] In view of above circumstances, an attempt was made to evaluate the nitrogen fixing potentiality of isolated heterocystous strains. Moreover, an attempt was also taken to correlate the nitrogen fixing capacity of the strains with some soil properties.

Materials and Methods

Thirty seven surface soil samples (0-5 cm) were collected from 26 districts of Bangladesh. Each was a composite sample of 5 sub-samples. Nitrogen free Fogg's medium[11] was used for isolation and consecutive experiments. Cyanobacterial strains were identified by standard texts[12,13] following their growth in the flasks and plates. Determination of pH, organic carbon[14], available phosphorus[15], total and available nitrogen of the soils and nitrogen fixation made by the cyanobacteria[16] was carried out by standard analytical techniques (Table 1-2). One ml of uniform suspension of 4 day old culture of each cyanobacterial strains was separately used to inoculate in 150 ml flasks containing 50 ml of sterilized Fogg's medium, in triplicate. Thirty day old cultures of cyanobacteria were used for the determination of nitrogen fixing capacity.

Results and Discussion

Results of nitrogen fixation capacity of 44 isolates of cyanobacteria collected from different soils and measured in liquid culture are presented in Table 1. A wide variation in the ability of nitrogen fixation was evident. The highest amount of nitrogen was fixed by *Nostoc commune* (5.41 mg 50 ml^{-1} 30 days) of Jhenaidah and that of the lowest (0.318 mg 50

Table 1. N_2-fixing capacity (mg 50 ml^{-1}) of the isolated cyanobacterial strains.

Serial no.	District	Soil samples	Isolate no.	Algal strain	Nitrogen fixation
1	Mirpur, Dhaka	1	1	*Nostoc linkia*	1.84
2	Brahmanbaria sadar	2	2	*Fischerella ambigua*	1.97
			3	*Nostoc linkia*	3.7
3	Maizdee, Noakhali	3	4	*Fischerella muscicola*	2.92
			5	*Aulosira aenigmatica*	2.94
4	Saintmartins, Coxsbazar	4	6	*Calothrix marchica*	4.48
5	Halishahar, Chittagong	5	7	*Fischerella sp.*	1.11
		5	8	*Fischerella muscicola*	1.38
		5	9	*Scytonema mirabile*	0.850
		6	10	*Nostoc paludosum*	1.84
		6	11	*Fischerella sp.*	1.21
		5	12	*Nostoc paludosum*	1.26
6	Rangamati sadar	7	13	*Calothrix marchica*	3.97
7	Khagrachari sadar	8	14	*Nostoc muscorum*	1.11
8	Adharshapara, Jhenaidah	9	15	*Nostoc commune*	5.41
		10	16	*Nostoc sp.*	2.19
9	Moheshkhali, Norail	11	17	*Nostoc linkia*	4.69
		11	18	*Anabaena variabilis*	3.91
10	Palash, Narshindi	12	19	*Calothrix marchica*	4.38
11	Mongalbaria, Kushtia	13	20	*Fischerella muscicola*	1.65
12	Naogaon, Rajshahi	14	21	*Fischerella sp.*	2.40
			22	*Hapalosiphon welwitschii*	3.31
13	Pabna sadar	15	23	*Calothrix crustacea*	2.08
			24	*Nostoc linkia*	3.0
14	Mohadebpur, Rangpur	16	25	*Hapalosiphon fontinalis*	0.53
15	Bhuapur, Tangail	17	26	*Hapalosiphon hibernicus*	1.58
		18	27	*Nostoc calcicola*	3.64
16	Fulbaria, Mymensingh	19	28	*Calothrix wembaerensis*	1.75
17	Zaflong, sylhet	20	29	*Stigonema sp.*	0.378
18	Churanaghat, Moulovibazar	21	30	*Nostoc paludosum*	2.12
			31	*Calothrix marchica*	2.54
19	Shariatpur sadar	20	32	*Nostoc sp.*	2.90
		20	33	*Nostoc carneum*	4.07
		21	34	*Hapalosiphon welwitschii*	1.26
20	Madaripur	22	35	*Fischerella muscicola*	2.54
21	Rajbari sadar	23	36	*Calothrix marchica*	2.15
		24	37	*Hapalosiphon hibernicus*	0.47
22	Paikkhali, Pirojpur	25	38	*Hapalosiphon welwitschii*	0.88
23	Jhalokathi sadar	26	39	*Nostoc carneum*	2.54
		27	40	*Fischerella muscicola*	1.58
24	Barisal sadar	28	41	*Nostoc verrucosum*	2.28
25	Khalishpur sadar, Khulna	29	42	*Nostoc ellipsosporum*	0.77
		30	43	*Nostoc sp.*	1.47
26	Morelgang, Bagerhat	31	44	*Hapalosiphon welwitschii*	1.12

Table 2. Some properties of the soil sample examined.

Sl. No.	District	pH	Org. matter%	Total N%	Available N(mg/100g)	Available P(µg/g)	C/N ratio
1	Mirpur, Dhaka	7.00	0.99	0.14	4.90	8.5	4.07
2	Brahmanbaria sadar	6.82	0.76	0.10	1.40	18.5	4.40
3	Maizdee, Noakhali	6.92	1.53	0.09	3.50	16.3	9.89
4	Saintmartins, Coxsbazar	6.68	0.38	0.11	1.40	10.2	2.00
5	Halishahar, Chittagong	6.84	1.26	0.11	1.40	21.4	6.44
	Farikchari, Chittagong	6.67	0.38	0.03	2.10	8.6	7.33
6	Rangamati sadar	7.22	0.38	0.06	3.50	17.5	3.67
7	Khagrachari sadar	6.20	1.27	0.13	9.80	10.1	5.70
8	Adharshapara, Jhenaidah	7.25	0.19	0.05	4.90	12.7	2.20
9	Moheshkhali, Norail	7.40	2.24	0.09	7.00	14.5	14.44
10	Palash, Narshindi	7.10	1.46	0.12	8.40	11.4	7.08
11	Mongalbaria, Kushtia	7.80	2.34	0.15	5.60	20.6	9.07
12	Naogaon, Rajshahi	6.50	1.72	0.10	5.10	13.0	10.00
13	Pabna sadar	7.80	0.32	0.06	5.60	15.3	3.17
14	Mohadebpur, Rangpur	5.70	1.36	0.08	8.40	14.6	9.88
15	Bhuapur, Tangail	6.90	2.73	0.19	2.80	6.5	8.37
	Mirjapur, Tangail	7.00	0.65	0.25	4.90	8.9	1.52
16	Fulbaria, Mymenshing	7.00	1.36	0.11	6.30	4.9	6.97
17	Zaflong, Sylhet	4.30	1.81	0.20	9.80	9.8	5.25
18	Churanaghat, Moulovibazar	7.20	0.65	0.08	2.10	16.5	4.75
19	Shariatpur sadar	7.20	0.76	0.11	5.60	8.8	4.00
	Ghosherhat, Shariatpur	7.50	0.65	0.13	3.50	15.5	2.92
20	Madaripur	7.80	0.38	0.15	4.90	8.5	1.47
21	Rajbari sadar	7.70	0.55	0.10	2.10	10.2	3.20
	Baliakandi, Rajbari	7.50	0.55	0.11	1.40	13.3	2.90
22	Paikkhali, Pirojpur	5.30	2.48	0.12	16.80	4.0	12.00
23	Jhalokathi sadar	6.00	2.65	0.10	11.90	16.0	15.9
	Rajapur Jhalokati	6.80	1.80	0.10	7.00	9.0	7.50
24	Barisal sadar	5.70	1.65	0.08	16.63	14.0	12.00
25	Khalishpur sadar, Khulna	6.80	1.48	0.11	12.78	3.0	7.80
	Alahipur, Rupsha, Khulna	6.60	1.07	0.07	7.70	6.7	8.86
26	Morelgang, Bagerhat	7.30	0.16	0.02	13.13	4.0	4.50

Table 3. Simple and multiple correlation co-efficients between N_2 -fixing capacity and soil properties.

Independent variable	Dependent variable	Co-efficient of correlation
pH	N_2-fixing capacity	0.3984 (P=0.01)
Organic matter	"	-0.2021 ns
Total N	"	-0.5521 (P=0.001)
Available N	"	-0.2611 (P=0.1)
Available P	"	0.3592 (P=0.02)
All variables	"	0.6101 (P=0.01)

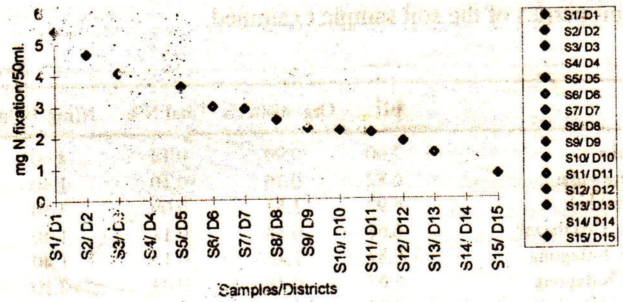

Fig. 1 Nitrogen fixing capacity of different species of *Nostoc* in 15 districts of Bangladesh.

Table showing used algal sample in districts according to the chart above.

	Used sample		*District*
1.	*N. commune*	1.	*Adarsapara, Jhenaidha*
2.	*N. linkia*	2.	*Moheshkhola, Norail*
3.	*N. carneum*	3.	*Sariatpur sadar*
4.	*N. linkia*	4.	*Brahmanbaria sadar*
5.	*N. calcicola*	5.	*Mirzapur, Tangail*
6.	*N. linkia*	6.	*Tinandina, Raigang, Sirajgang*
7.	*N. sp.*	7.	*Sariatpur sadar*
8.	*N. carneum*	8.	*Jhalokati sadar*
9.	*N. verrucosum*	9.	*Barisal sadar*
10.	*N. sp.*	10.	*Adarsapara, Jhenaibha*
11.	*N. paludosum*	11.	*Churanaghat, Moulovibazar*
12.	*N. paludosum*	12.	*Fatikchari and Mirpur, Dhaka*
13.	*N. sp.*	13.	*Rupsha. Khulna*
14.	*N. mascorum*	14.	*Khagrachori sadar*
15.	*N. ellipsosporum*	15.	*Khalishpur sadar, Khulna*

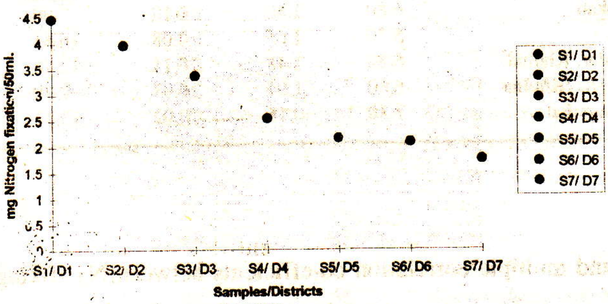

Fig.2. Nitrogen fixing capacity of different species of *Calothrix* in 7 districts of Bangladesh.

Table showing used algal sample in districts according to the chart above.

	Used sample name	**District**
1.	*C. marchica*	Cox's bazar
2.	*C. marchica*	Rangamati sadar
3.	*C. marchica*	Palash, Narshindi
4.	*C. marchica*	Churanaghat, Moulovibazar
5.	*C. marchica*	Rajbari sadar
6.	*C. crustacea*	Sirajgang
7.	*C. wembaerensis*	Fulbaria, Mymensingh

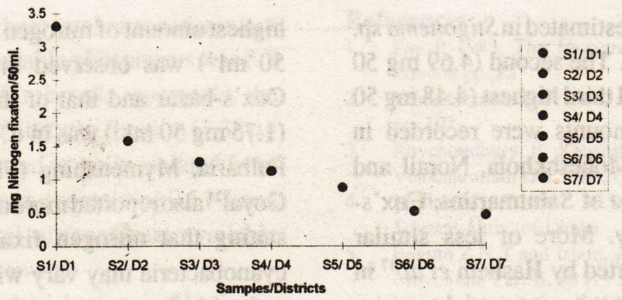

Fig. 3 Nitrogen fixing capacity of different species of *Hapalosiphon* in 7 districts of Bangladesh.

Table showing used algal sample in districts according to the chart above.

	Used sample	District
1.	*H. welwitschii*	Naogaon, Rajshahi
2.	*H. hibernicus*	Bhuapur, Tangail
3.	*H. welwitschii*	Ghoserhat, Shariatpur
4.	*H. welwitschii*	Kakdanga, Fakirhat, Bagerhat
5.	*H. welwitschii*	Paikkhali, Bhandaria, Pirojpur
6.	*H. fontinalis*	Mohadebpur Rangpur
7.	*H. hibernicus*	Baliakandi, Rajbari

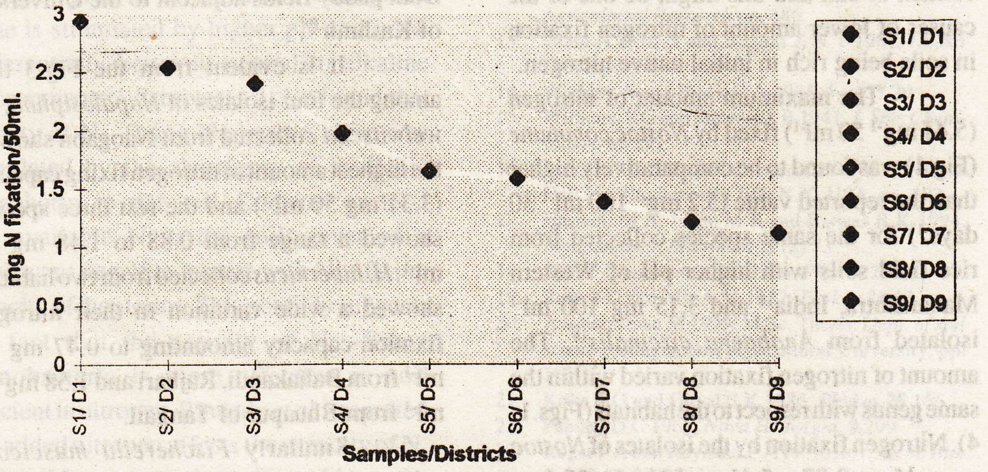

Fig.4. Nitrogen fixing capacity of different species of *Fischerella* in 9 districts of Bangladesh.

Table showing used algal sample in districts according to the chart above.

	Used sample		District
1.	*F. muscicola*	1.	Maizdee, Noakhali
2.	*F. muscicola*	2.	Madaripur
3.	*F. sp.*	3.	Naogaon, Rajshahi
4.	*F. ambigua*	4.	Brahmanbaria sadar
5.	*F. muscicola*	5.	Mangalbaria, Kushtia
6.	*F. muscicola*	6.	Rajapur, Jhalokati
7.	*F. muscicola*	7.	Halishahar, chittagong
8.	*F. sp.*	8.	Fatikchari, Chittagong
9.	*F. sp.*	9.	Halishahar, Chittagong

ml^{-1} 30 days^{-1}) was estimated in *Stigonema* sp. of Zaflong, Sylhet. The second (4.69 mg 50 ml^{-1} in 30 days) and third highest (4.48 mg 50 ml^{-1} 30 days^{-1}) amounts were recorded in *Nostoc linkia* of Moheshkhola, Norail and *Calothrix marchica* of Saintmartins, Cox's-bazar respectively. More or less similar variation was reported by Hashem *et al.*[17] in nitrogen fixation capacity among eight species of *Nostoc* isolated from different agro-ecological zones of Bangladesh. The relatively higher amount of nitrogen fixation in the soils might be due to the lower content of total nitrogen (i.e. 0.05, 0.09 and 0.11% of the soil respectively. Mandal *et al.*[9] also reported that the quantity of nitrogen fixing blue-green algae varied negatively with total nitrogen content of soil and this might be one of the causes of lower amount of nitrogen fixation in soils being rich in initial native nitrogen.

The maximum amount of nitrogen (5.41 mg^{-1} 50 ml^{-1}) fixed by *Nostoc commune* (Fig.1) was found to be comparatively higher than the reported value (5.2 mg^{-1} 100 ml^{-1} 30 days^{-1}) for the same species collected from rice field soils with higher pH of Western Maharashtra, India[18] and 3.15 mg 100 ml^{-1} isolated from *Anabaena circinalis*[19]. The amount of nitrogen fixation varied within the same genus with respect to the habitats (Figs.1-4). Nitrogen fixation by the isolates of *Nostoc* ranged from 0.77 to 5.41 mg 50^{-1} ml at 30 days of incubation. These findings agreed favourably well with the earlier reports of Asaduzzman[20] and Kolte and Goyal[21]. The amount of nitrogen fixation, 2.12 mg 50 ml^{-1} by *Nostoc paludosum* at Moulovibazar seems to be close to the value (2.47 mg 50 ml^{-1}) reported for the same species isolated from Vidarbha region of Maharashtra[21].

In case of *Calothrix* isolates, the highest amount of nitrogen fixation (4.48 mg 50 ml^{-1}) was observed in *C. marchica* of Cox's-bazar and that of the lowest amount (1.75 mg 50 ml^{-1}) was in *C. wembaerensis* of Fulbaria, Mymenshing (Fig. 2). Kolte and Goyal[21] also reported more or less same results stating that nitrogen fixation capacity of cyanobacteria may vary widely with genera and with the species of the same genus. In their study as well the highest amount of nitrogen fixation was recorded in *Calothrix marchica* and that of lowest was observed in *C. wembaerensis* among the seven species of the genus. The amount of nitrogen fixed (4.48 mg 50 ml^{-1}) by *C. marchica* was also comparatively higher than that recorded for *Gloeotrichia echinulata* (2.98 mg 100 ml^{-1}) from paddy fields adjacent to the University of Kashmir[19].

It is evident from the Fig.3 that among the four isolates of *Hapalosiphon*, *H. welwitschii* collected from Naogaon showed the highest amount of nitrogen fixing capacity (3.31 mg 50 ml^{-1}) and the rest three species showed a range from 0.88 to 1.48 mg 50 ml^{-1}. *H. hibernicus* collected from two habitats showed a wide variation in their nitrogen fixation capacity amounting to 0.47 mg 50 ml^{-1} from Baliakandi, Rajbari and 1.58 mg 50 ml^{-1} from Bhuapur of Tangail.

Similarly *Fischerella muscicola* collected from 6 different districts also varied widely among themselves in the nitrogen fixation capacity (Fig.4). Maximum amount (2.92 mg 50 ml^{-1}) was obtained in the strains isolated from Maizdee of Noakhali where the soil was poor in total nitrogen (0.09%) and relatively rich in available phosphorus (16.3 µg gm^{-1}). The second highest amount of nitrogen fixation (2.54 mg 50 ml^{-1}) was estimated in *Fischerella muscicola* isolated from Madaripur where total nitrogen content was 0.15% and available phosphorus was 8.5 μg^{-1} soil. Among *Fischerella muscicola,* the minimal amount of nitrogen fixation (1.38 μg 50 ml^{-1}) was measured in strain isolated from Halishahar, Chittagong containing 0.11% and 21.4 μg g^{-1} of total nitrogen and available phosphorus respectively.

Relationship between N_2-fixing capacity and soil properties has been assessed (Table 3). Simple correlation suggests that N_2 -fixing capacity of the cyanobacterial strains significantly increased with the decrease in acidity and increase in available phosphorus content of the soil. These findings are in good agreement with the observations of other investigators[22] who proposed that optimum growth of N_2 fixing blue-green algae is stimulated by higher pH and grow preferentially better in neutral to alkaline environments[23]. Srinivasan[24] also found that activity of N_2 -fixing blue-green algae was stimulated by the abundance of available phosphorus in rice soil. Contents on total and available N in the soils revealed a significant negative impact with N_2 - fixing capacity of the strains. This possibly suggests the fact that the activity of N_2 -fixing cyanobacteria is more effective in soil deficient in nitrogen. Rinaudo *et al.*[25] reported that added nitrogen affects the capacity of N_2 -fixing blue-green algae in paddy soils. Content of organic matter showed no significant impact on nitrogen fixation capacity by cyanobacterial strain.

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