

Stress compatability in cyanobacteria

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Abstract

The importance of diazotrophic cyanobacteria in maintaining the spontaneous fertility of the rice field soil has been well documented. The present day need is to develop region specific strains of cyanobacteria capable of carrying out the desired functions under different agro-climatic conditions. In this context the distributional pattern and comparative predominance of different cyanobacterial forms has been worked out for 142 rice field soil samples collected from Kurnool district of Andhra Pradesh, India. Total 72 algal forms were recorded in the enrichment cultures of the soil samples, out of these only 5 were greens and the rest were blue-greens. During the studies, 42 unialgal isolates belonging to 8 genera were obtained and screened for growth and nitrogen fixation. A remarkable strain variation was observed even at intra-species level. Although, *Nostoc* and *Anabaena* had comparatively a wider distribution than *Calothrix* the later was found to perform consistently better both in terms of growth and nitrogen fixation. Presence of ammonium nitrogen had a differential effect on growth and nitrogen fixation. The threshold value for nitrogen fixation was found to be much lower than that for growth but even at 100 $\mu\text{g NH}_4\text{N/ml}$, in none of the algae, the process of nitrogen fixation was completely stopped. Implications of this property in increasing the availability of biologically fixed nitrogen as well as fertilizer nitrogen have been emphasized.

Introduction

- **Kurnool district of Andhra Pradesh in India is situated within the geographical coordination of 14⁰-54' and 16⁰-11' of the northern latitude and 76⁰ -58' and 78⁰ -25' of the eastern latitude, average elevation is 33.33 meters m.s.l. with tropical climate.**
- **Temperature in summer goes upto 46.1⁰ C and in winter it ranges from 15.5 to 32.2⁰ C. unevenly distributed rainfall with annual average varying from 610 to 620mm, rice is the main cultivated crop both as rainfed and upland conditions.**
- **Diazotrophic cyanobacteria are recognized as the most potential nitrogen fixing biological system under waterlogged conditions of the rice fields**
- **Distributional pattern of cyanobacteria**
- **Natural potential of growth and nitrogen fixation.**
- **Effect of graded concentrations of ammonical nitrogen on growth and nitrogen fixation**

Material and Methods

- One hundred and forty two randomized soil samples were collected from the fallow rice fields of Kurnool, Andhra Pradesh.
- The enrichment cultures were raised separately in Fogg's medium with and without potassium nitrate
- Forty two cyanobacterial forms were isolated and tested for their natural potential of growth and nitrogen fixation.
- Out of these 15 promising strains were selected to observe the effect of graded concentration of ammonical nitrogen on growth and nitrogen fixation.
- Algal isolates were grown in triplicate with and without 25, 50, 75 and 100 $\mu\text{g NH}_4^+\text{Nml}^{-1}$ supplied as ammonium chloride.
- The flasks were inoculated with 1 ml of homogenized 10 day old growth of each alga and incubated for 28 days at 2000 lux light intensity with 16/8 h light/dark cycle and $30 \pm 2^\circ\text{C}$ temperature. Initial observations on growth and total nitrogen were taken at 0 day. On completion of the experiment the contents were homogenized and volume made up to 50 ml. From each replication, two 10ml portions were taken for chlorophyll estimation (McKinny, 1941) and the rest were used for recording dry weight. After weighing, the filter paper with the algal mat and the filtrate were together used for estimating total nitrogen (Jackson, 1958).

Results

- A total of 72 algal forms were recorded out of which 67 were cyanobacteria. The cyanobacteria belonged to 16 genera and the 5 green algal genera were represented by one species each (Table 1).
- Judged by the rated score *Nostoc*, *Anabaena* and *Calothrix* were found to be very widely distributed. Forms like *Phormidium*, *Aphanothece*, *Cylindrospermum*, *Hapalosiphon*, *Plectonema* and *Westiellopsis* were sparsely distributed. *Aphanocapsa*, *Chloroglea*, *Gleocapsa*, *Microchaete* and *Microcoleus* had a highly restricted distribution (Table 2).
- Among the 42 isolates tremendous variation was observed in growth and nitrogen fixation not only between different genera and species but also within different isolates of the same species (Table 3)
- The promising 15 cyanobacteria in general, showed a concentration dependant decrease in all the three parameters except in *Anabaena sphaerica* 19, *Hapalosiphon hibernicus* 111 and *Microchaete tenera* 76 (Table 4).

TABLE 1. Frequency distribution of different algal genera.

Genus	No. of species	Species
<i>Nostoc</i>	20	<i>muscorum, punctiforme, ellipso sporum, linckia, piscinale, calcicola, hatei, passerinianum, carneum, microscopicum, sphaericum, verrucosum, paludosum, commune, humifusum, rivulare, spongiaeforme, entophytum</i>
<i>Anabaena</i>	14	<i>laxa, fertilissima, iyengarii, anomala, variabilis, oryzae, doliolum, spiroides, torulosa, orientalis, sphaerica, gelatinicola, khannae, ambigua</i>
<i>Calothrix</i>	6	<i>marchica, brevissima, javanica, epiphytica, membranacea, elenkinii</i>
<i>Cylindrospermum</i>	4	<i>muscicola, licheniformae, stagnale, majus</i>
<i>Phormidium</i>	1	<i>faoveolarum</i>
<i>Oscillatoria</i>	6	<i>subbrevis, nigra, sancta, martinii, obscura, limosa</i>
<i>Hapalosiphon</i>	5	<i>intricatus, fontinalis, hibernicus, stuhlmanni, welwitschii</i>
<i>Plectonema</i>	1	
<i>Aphanothece</i>	1	
<i>Westiellopsis</i>	1	<i>prolifera</i>
<i>Scytonema</i>	3	<i>bohneri, schmidtii, malviyaensis</i>
<i>Microcheate</i>	1	<i>tenera</i>
<i>Gloeocapsa</i> sp.	1	
<i>Aphanocapsa</i> sp.	1	
<i>Chloroglea</i> sp.	1	
<i>Microcoleus</i> sp.	1	
<i>Chlorococcum</i>	1	
<i>Closterium</i>	1	
<i>Oedogonium</i>	1	
<i>Rhizoclonium</i>	1	
<i>Ulothrix</i>	1	

The species and genera listed in order of their frequency occurrence on their rated score.

TABLE 2. Comparative distribution of algal genera in Kurnool district of Andhra Pradesh.

Genus	No. of species	Numerical total	Rated total	Rated score
<i>Nostoc</i>	20	459	1635	1151.4
<i>Anabaena</i>	14	365	1426	1004.2
<i>Calothrix</i>	6	231	845	595.0
<i>Cylindrospermum</i>	4	118	492	346.2
<i>Phormidium</i>	1	132	438	308.2
<i>Oscillatoria</i>	6	104	287	202.1
<i>Hapalosiphon</i>	5	66	243	171.1
<i>Plectonema</i>	1	69	229	161.2
<i>Aphanothece</i>	1	42	151	106.3
<i>Westiellopsis</i>	1	23	93	65.4
<i>Scytonema</i>	3	15	50	35.2
<i>Microcheate</i>	1	14	27	19.0
<i>Gloeocapsa</i>	1	7	22	15.4
<i>Aphanocapsa</i>	1	6	14	9.8
<i>Chloroglea</i>	1	5	12	8.2
<i>Microcoleus</i>	1	5	6	4.2
<i>Chlorococcum</i>	1	142	527	371.1
<i>Closterium</i>	1	21	50	35.2
<i>Oedogonium</i>	1	28	92	64.7
<i>Rhizoclonium</i>	1	28	111	78.1
<i>Ulothrix</i>	1	14	51	35.9

TABLE 3. Growth and nitrogen fixation by cyanobacterial isolates.

Isolate No.	Name of the isolate	Chlorophyll	Dry weight	Total nitrogen
93	<i>Anabaena gelatinicola</i>	2.78	100.00	30.80
19	<i>A. sphaerica</i>	5.40	166.60	43.02
21	<i>A. sphaerica</i>	4.06	390.00	41.02
77	<i>A. torulosa</i>	1.26	90.00	30.80
119	<i>Calothrix elenkinii</i>	1.08	40.00	35.70
3	<i>C. epiphytica</i>	4.48	406.00	54.60
96	<i>C. epiphytica</i>	5.79	460.00	44.61
104	<i>C. epiphytica</i>	11.19	486.60	69.30
32	<i>C. javanica</i>	6.57	446.60	57.40
3a	<i>C. marchica</i>	3.80	113.30	34.72
6	<i>C. marchica</i>	5.65	220.00	41.72
100	<i>C. marchica</i>	5.49	433.30	63.70
106	<i>C. marchica</i>	6.58	446.60	55.20
6a	<i>C. membranacea</i>	5.03	226.60	51.10
74	<i>Cylindrospermum muscicola</i>	2.31	353.30	50.23
81	<i>C. muscicola</i>	5.39	580.00	51.80
111	<i>Hapalosiphon hibernicus</i>	4.11	186.60	34.30
12	<i>H. intricatus</i>	3.90	220.00	32.80
76	<i>Microchaete tenera</i>	4.50	286.60	51.80

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Isolate No.	Name of the isolate	Chlorophyll	Dry weight	Total nitrogen
23	<i>Nostoc carneum</i>	3.49	413.30	47.88
87	<i>N. ellipsosporum</i>	0.64	140.00	23.10
88	<i>N. entophytum</i>	1.26	80.00	26.36
82	<i>N. linckia</i>	0.63	66.60	25.20
91	<i>N. linckia</i>	0.52	46.60	17.26
105	<i>N. microscopicum</i>	0.55	93.30	23.10
15	<i>N. muscorum</i>	5.52	246.60	44.98
42	<i>N. muscorum</i>	2.29	146.60	31.03
92	<i>N. muscorum</i>	4.98	513.30	54.88
103	<i>N. muscorum</i>	4.68	473.30	48.16
20	<i>N. paludosam</i>	4.30	313.30	43.30
99	<i>N. paludosam</i>	2.66	346.60	40.60
43	<i>N. passerinianum</i>	6.99	420.00	72.80
26	<i>N. punctiforme</i>	5.67	450.00	49.70
46	<i>N. punctiforme</i>	3.72	280.00	36.40
102	<i>Nostoc sp.</i>	1.65	40.00	24.50
122	<i>N. spongiaeformae</i>	1.93	86.60	27.30
41	<i>N. verrucosum</i>	1.26	60.00	23.80
4a	<i>Scytonema schmidtii</i>	6.75	373.30	43.16
30	<i>Westiellopsis prolifica</i>	2.43	106.60	27.25
47	<i>W. prolifica</i>	3.37	86.60	35.00
80	<i>W. prolifica</i>	5.92	453.30	46.62
98	<i>W. prolifica</i>	0.94	73.30	20.30

Values in $\mu\text{g/ml}$

TABLE 4. Effect of ammonium nitrogen on growth and nitrogen fixation by Cyanobacteria

(Conc. $\mu\text{g ml}^{-1}$)	Chloro- phyll	Per cent Change	Dry. Wt	Per cent Change	Total N 0 day	Total N 28 day	Per cent Change
<i>Anabaena sphaerica</i> (19)							
0	4.90	—	501.0	—	5.98	52.43	—
25	5.42	+ 10.6	510.0	+ 1.7	20.33	53.11	- 29.4
50	6.43	+ 31.2	420.0	- 16.1	40.00	55.55	- 66.5
75	6.02	+ 22.8	405.0	- 19.0	60.33	72.34	- 74.1
100	5.03	+ 2.6	365.0	- 27.1	80.00	92.27	- 73.5

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(Conc. $\mu\text{g ml}^{-1}$)	Chloro- phyll	Per cent Change	Dry. Wt	Per cent Change	Total N 0 day	Total N 28 day	Per cent Change
<i>Calothrix epiphytica</i> (3)							
0	5.16	—	399.0	—	2.33	41.66	—
25	2.76	- 46.5	283.0	- 29.1	20.00	43.78	- 39.6
50	2.62	- 49.2	249.0	- 37.6	40.33	48.59	- 79.8
75	1.75	- 66.1	201.0	- 49.6	60.00	68.28	- 78.9
100	1.70	- 67.1	199.0	- 50.1	80.33	82.74	- 93.9
<i>Calothrix epiphytica</i> (104)							
0	5.75	—	483.0	—	3.00	46.50	—
25	3.78	- 34.2	183.0	- 62.1	20.00	48.25	- 35.0
50	3.88	- 32.5	183.0	- 62.1	40.00	59.88	- 54.3
75	3.12	- 45.7	183.0	- 62.1	60.00	67.25	- 83.3
100	3.00	- 47.8	233.0	- 51.7	80.00	87.27	- 83.3
<i>Calothrix javanica</i> (32)							
0	6.36	—	448.7	—	5.00	55.76	—
25	5.39	- 15.2	284.0	- 36.7	20.00	58.16	- 24.8
50	5.08	- 20.1	236.0	- 47.4	41.00	65.80	- 51.1
75	4.54	- 28.6	185.0	- 58.7	60.33	62.52	- 95.7
100	3.58	- 43.7	166.0	- 63.0	30.33	84.22	- 92.3
<i>Calothrix marchica</i> (100)							
0	6.14	—	483.0	—	3.00	45.64	—
25	2.36	- 61.6	266.0	- 44.9	20.00	47.16	- 36.3
50	2.71	- 55.9	198.7	- 58.9	40.33	50.11	- 77.1
75	2.30	- 62.5	172.3	- 64.3	60.33	62.83	- 94.1
100	1.98	- 67.8	170.0	- 64.8	80.33	82.12	- 95.8
<i>Calothrix marchica</i> (106)							
0	6.39	—	466.2	—	3.00	45.79	—
25	1.73	- 73.0	249.1	- 46.6	20.00	44.16	- 43.5
50	1.14	- 82.1	216.2	- 53.7	40.33	47.90	- 82.3
75	1.77	- 72.3	183.0	- 60.7	60.33	62.39	- 95.2
100	1.59	- 75.1	166.0	- 64.4	80.33	82.14	- 95.8
<i>Calothrix membranacea</i> (6)							
0	4.06	—	400.5	—	3.00	45.26	—
25	1.37	- 66.2	266.0	- 28.6	20.33	50.48	- 28.7
50	1.05	- 74.1	199.7	- 50.1	40.33	51.27	- 74.1
75	0.91	- 77.6	199.0	- 50.3	60.33	72.48	- 71.2
100	0.71	- 82.5	166.0	- 58.2	80.33	89.45	- 78.4
<i>Cylindrospermum muscicola</i> (81)							
0	5.53	—	416.3	—	4.00	46.23	—
25	3.14	+ 43.2	236.0	- 43.3	20.00	48.27	- 33.1
50	2.01	+ 63.7	167.5	- 59.8	40.00	51.78	- 72.1
75	1.99	+ 64.0	166.0	- 60.1	61.00	63.73	- 93.5
100	1.80	- 67.5	163.0	- 60.9	80.00	81.82	- 95.7

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(Conc. $\mu\text{g ml}^{-1}$)	Chloro- phyll	Per cent Change	Dry. Wt	Per cent Change	Total N 0 day	Total N 28 day	Per cent Change
<i>Hapalosiphon hibernicus</i> (111)							
0	3.81	—	349.7	—	7.00	33.27	—
25	5.12	+ 34.4	399.8	+ 14.3	20.33	35.50	- 42.3
50	5.70	+ 49.6	433.3	+ 23.9	40.33	42.68	- 91.1
75	4.51	+ 18.4	333.3	- 4.7	60.33	61.44	- 95.8
100	4.75	+ 24.9	250.2	- 28.5	80.00	82.33	- 91.2
<i>Microchaete tenera</i> (76)							
0	5.02	—	355.0	—	4.02	46.84	—
25	6.93	+ 38.1	399.0	+ 12.4	20.33	48.49	- 34.2
50	6.66	+ 32.7	267.0	- 24.6	40.33	59.85	- 54.4
75	5.26	+ 4.8	234.0	- 33.9	61.00	73.62	- 70.5
100	4.22	- 15.9	166.0	- 53.2	80.33	83.53	- 92.5
<i>Nostoc muscorum</i> (92)							
0	4.12	—	316.0	—	4.00	50.66	—
25	2.77	- 32.8	220.3	- 30.3	20.00	53.68	- 27.4
50	2.91	- 29.4	220.3	- 30.3	40.00	64.29	- 47.9
75	2.39	- 41.9	200.0	- 36.7	60.00	78.20	- 61.0
100	2.11	+ 48.8	202.2	- 36.0	80.00	86.40	- 86.3
<i>Nostoc passerinianum</i> (43)							
0	4.56	—	202.2	—	3.00	49.40	—
25	1.03	- 77.4	183.0	- 9.5	20.00	54.23	- 26.2
50	1.12	- 75.4	166.5	- 17.6	40.00	58.61	- 59.9
75	1.47	- 67.8	166.0	- 17.9	60.00	71.00	- 78.5
100	1.66	- 63.6	149.3	- 26.1	80.00	88.43	- 81.8
<i>Nostoc punctiforme</i> (26)							
0	6.73	—	584.0	—	5.00	56.50	—
25	4.00	- 40.5	333.0	- 43.0	20.00	54.07	- 33.8
50	2.62	- 61.0	216.0	- 63.0	40.00	45.20	- 89.9
75	2.58	- 61.6	202.2	- 65.4	60.00	71.50	- 77.7
100	1.42	- 78.9	183.0	- 68.7	80.00	83.00	- 94.2
<i>Scytonema schmidtii</i> (4a)							
0	5.90	—	366.0	—	3.00	42.34	—
25	1.10	- 81.4	216.3	- 41.0	20.33	45.38	- 36.3
50	1.25	- 78.8	183.3	+ 49.9	40.00	48.50	- 77.8
75	0.99	- 83.2	199.7	+ 45.5	60.00	66.78	- 88.8
100	0.59	- 90.0	168.5	+ 54.1	80.33	89.82	- 75.9
<i>Westiiopsis prolifica</i> (80)							
0	5.90	—	416.3	—	3.16	48.18	—
25	3.42	- 42.0	336.3	- 19.2	20.33	49.96	- 34.2
50	3.22	- 45.4	269.5	- 35.3	40.33	42.68	- 94.8
75	2.76	- 53.2	266.7	- 36.0	60.33	62.68	- 94.8
100	2.76	- 53.2	283.0	- 32.0	80.33	81.89	- 96.5

Values in $\mu\text{g ml}^{-1}$

Discussion

- Kolte and Goyal (1985) reported *Calothrix* as one of the dominant cyanobacteria from Maharashtra state, India. In the present study also *Calothrix* turned out to be the third most widely distributed genus in Kurnool district of Andhra Pradesh. Forms like *Calothrix*, *Tolypothrix*, *Aulosira* and *Scytonema* which are capable of growing luxuriantly on moist and shaded soil surfaces, attain importance as inoculants under upland paddy conditions where the waterlogged conditions cannot be maintained permanently.
- Existence of remarkable variation even at intraspecies level in the nitrogen fixing ability and biomass production has thrown open the possibility of selecting the desired strains through screening of the autochthonous cyanobacteria.
- Kolte and Goyal (1986) observed that species of *Calothrix*, *Tolypothrix*, *Aulosira*, *Cylindrospermum*, *Hapalosiphon* and *Westiellopsis* and opined that these forms capable of growing on moist and shaded soil surface, hold promise for algalization of upland paddies.
- Urea and ammonium sulphate are the commonly used fertilizers, having nitrogen in NH_4^+ form which directly affects both growth and nitrogen fixation by cyanobacteria. NH_4^+ N is a putative inhibitor of nitrogen fixation.

- **The differential response of growth and nitrogen fixation to the presence of $\text{NH}_4^+ \text{N}$ has interesting implications in the algal biofertilizer technology. Because of higher threshold value for growth, coupled with the application of fertilizer nitrogen in split doses and dilution of the effect due to various soil components, it appears that under field conditions,**
- **$\text{NH}_4^+ \text{N}$ may never limit the biomass production by the cyanobacteria. Even if at these reduced levels of fertilizer nitrogen, the contribution of biologically fixed nitrogen may be considerably reduced, the loss may be more than compensated when the increased algal biomass reverts back to nitrogen fixation due to the progressive fall in the concentration of fertilizer nitrogen.**
- **This will not only provide increased amount of biologically fixed nitrogen but also increase the availability of part of the fertilizer nitrogen by preventing it from being lost through metabolization by the algae under non-nitrogen fixing conditions.**
- **The remarkable variation in growth and nitrogen fixing potential of different cyanobacteria perceptible even at intra species level suggests that screening of natural populations can lead to the identification of algal strains making ideal inoculants compatible with higher level of fertilizer nitrogen.**