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Rhizosphere dynamics of inoculated cyanobacteria and their growth-promoting role in rice crop

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Abstract

Nitrogen fixing cyanobacteria are the predominant flora in waterlogged paddy fields which contribute significantly towards nitrogen budgeting in these ecosystems. Their establishment and role in plant growth promotion and soil microbial activity is poorly known. Under greenhouse conditions, pots were inoculated with one of a set of twenty cyanobacterial strains isolated from the rhizosphere of diverse rice and wheat varieties. Several strains established in the soil and persisted up to the harvest stage in soil and roots, significantly enhancing soil microbial biomass carbon, available nitrogen, and related soil microbiological parameters, and increased grain yields and grain weight. This can help in selecting promising strains for developing carrier-based inoculants to promote the growth of crop and soil microflora, leading to enhanced soil fertility and crop yields.

Keywords: cyanobacteria; rice rhizosphere; soil fertility

Introduction

Cyanobacteria comprise a large group of structurally complex and ecologically significant gram-negative prokaryotes, which exhibit a wide range of nutritional capabilities ranging from obligate phototrophy to heterotrophy (Rippka 1972, Vasudevan *et al*. 2006, Prasanna *et al*. 2008), although the majority of forms examined so far exhibit phototrophy. They live wherever there is light in a wide range of terrestrial, freshwater and hypersaline environments, among which soil is the best-studied terrestrial habitat. Cyanobacteria are a remarkable group of prokaryotes, which are known to exist independently and in symbiotic/facultative associations with a diverse range of members of the plant kingdom, including Gymnosperms, Pteridophytes and Bryophytes (Rai & Bergman 2002). However, their associations with crop plants are less explored (Nilsson *et al*. 2005). They are well adapted to a wide range of environmental conditions **a**nd have been widely employed as inoculants for enhancing soil fertility and improving soil structure, besides enhancing crop yields, especially in rice (Venkataraman 1972, Kaushik 2004, Nayak *et al*. 2004, Dhar *et al*. 2007). Most of the studies reported in literature do not provide in-depth information regarding the mode of action involved in plant growth stimulation, and only report stimulation of growth yields (Venkataraman & Neelakantan 1967, Misra & Kaushik 1989, Karthikeyan *et al.* 2007)

The paddy-field ecosystem represents a unique aquatic-terrestrial habitat, which provides a favorable environment for growth of and nitrogen fixation by cyanobacteria, meeting their requirements for light, water, elevated temperature and nutrient availability. This, in turn, is considered to be one of the major reasons for the relatively stable yield of rice under flooded conditions and maintenance of the productivity of rice fields (Roger *et al*. 1993). Cyanobacteria also add organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter content of the soil, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants (Mandal *et al.* 1998, Kaushik 2004)

Plant roots and the rhizosphere offer a suitable niche for most microorganisms, but the proliferation of cyanobacteria has not been well investigated (Prasanna *et al.* 2008). Enhancement of rice seed germination, root and shoot growth, weight of rice grains and their protein content and the fertilizing action of N_2-fixing cyanobacteria has been generally

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attributed to the release of synthesized nitrogenous compounds either by decomposition of the cells or excretion (Venkataraman & Neelakantan 1967, Nayak *et al*. 2004). Most researchers are of the opinion that the growth-promoting substances produced by cyanobacteria may be hormones *i.e.* like auxins, gibberellins, cytokinins or abscisic acid, vitamins or amino acids. Antibiotic or toxic compounds and molecules with pharmacological, immuno-suppressive or enzyme-inhibiting activities have also been reported (Namikoshi & Rinehart 1996, Sergeeva *et al*. 2002). However, some of the major limitations for the widespread use of cyanobacterial biofertilizers have been the scanty information available on their multifaceted nature (besides their well-established role as diazotrophs), establishment proficiency in diverse rice soil ecologies and ability to compete with the native flora and fauna.

Our investigation was aimed therefore towards evaluating the rhizosphere dynamics of inoculated cyanobacterial strains, their plant-growth promoting ability and effect on soil fertility in a pot culture experiment carried out under greenhouse conditions.

Materials & Methods

A pot culture experiment was designed to evaluate the effect of a set of selected cyanobacterial isolates on the yield of rice (variety Pusa Basmati 1) under glasshouse conditions, using unsterile soil taken from IARI fields (at 28° 4’ N, 77° 12’ E, altitude 228 m above sea level). The physico-chemical properties of the soil were as follows: pH 7.3, EC 0.36 dSm^{-1}, available nitrogen 87.5 kg ha^{-1}, available phosphorus 18.5 kg ha^{-1}, a sandy clay loam classified as a Udic Ustocrept of the Inceptisols. The recommended rate of fertilizer for rice is 120:80:60 NPK kg ha^{-1}. Treatments consisted of 12" plastic pots each filled with 12 kg of soil and inoculated with single isolate combinations together with one-third the nitrogen and the full dose of phosphate and potassium fertilizers, replicated three times. Uninoculated controls involved just fertilizers at three different levels (see Table 1). Pots were irrigated regularly, maintaining them at 60% of the water-holding capacity of the soil.

Rice seedlings 3-weeks old were transplanted into each pot (5 seedlings per pot) with equal spacing and irrigation was given according to routine agronomic practices. Fertilizer was applied at the time of transplantation, but in the control treatments involving the full- (treatment 23 of Table 1) and 2/3-dose N treatments (treatment 22 of Table 1), one-third of the N was given at the start, and the remainder at the tillering stage. Each treatment consisted of the inoculation of one of a set of 20 cyanobacterial strains (see Table 1) isolated from the rhizosphere of different rice varieties from diverse agro-ecological situations in India. The cyanobacterial strains belong to the germplasm of axenized unicyanobacterial isolates (Prasanna *et al*. 2006, 2009). Strain identification used taxonomic keys following careful observations related to the growth pattern on agar and in liquid media, and microscopic examination of color, shape and size of cells/filaments. Suspensions of log-phase (12-14 d) cultures of selected cyanobacterial isolates (prepared by high speed centrifugation at 8000 rev min^{-1} for 10 min, followed by washing and dissolution of the pellet using sterile water) were applied twice (5 and 30 d after transplanting) at the rate of 5 µg chlorophyll g^{-1} per pot. In order to evaluate the rhizosphere-occupying competence of these isolates, soil cores were removed near the root region, and the culture suspensions poured into the resulting pit and covered with soil.

Three samples were analysed: a) soil samples from 0-20 cm depth, collected at the harvest stage; b) rhizosphere soil, the soil adhering to the roots after careful uprooting of the plants; and c) the roots themselves, carefully excised and washed gently in running water and repeatedly with sterile water to remove any adhering soil or other particulate matter.

The soil samples were air-dried, mixed well and stored at -5 °C. Cyanobacteria were estimated in the soil samples from 0-20 cm depth by adopting the “most probable number”

(MPN) technique using a modified 96-well titre plate assay (Prasanna *et al.* 2006). The standard BG-11 medium was supplemented with cycloheximide (Sigma Chemical Co., USA) at a concentration of 50 µg ml^{-1} for the elimination of eukaryotes. All titer plates were sealed with Reynolds 900 film punctured with tiny holes for air circulation and incubated under controlled conditions of temperature (27 ± 2 ^{o}C) and light intensity (3000 lux); the number of wells showing visible growth at each dilution was counted after 2 weeks, and the MPN calculated from a standard table.

The roots were cut into 1-2 cm sections and inoculated into liquid BG-11 medium (devoid of nitrogen). The enrichment tubes with rhizosphere soil, 0-20 cm depth soil, root bits and MPN titre plates were regularly monitored for growth and observed microscopically. Standard plating/streaking techniques were used for isolation and purification of cyanobacterial strains (Stanier *et al.* 1971). Colonies and growth were regularly examined microscopically under a Nikon (Microphot-FX) light microscope and the nature of filaments, shape and size of vegetative cells, heterocysts and akinetes, measured; strain identification was done using the taxonomic keys of Desikachary (1959). Behaviour in culture and morphological examination of the selected set of strains was carried out at different stages of growth in the BG-11 medium, in liquid and solid (agar) media. The cyanobacterial strains identified from enrichment cultures raised for the samples taken at the beginning of the experiment and harvest stage from the uninoculated control pots (T21-23) served as referral points for evaluating the establishment proficiency of the inoculated strains.

To determine the carbon of the microbial biomass, pre-weighed freshly removed soil (approx. 35 g dry weight) was taken in Schott bottles. To one set of bottles, 2 ml of ethanol-free chloroform was added, the lid closed tightly, and the bottle shaken vigorously to mix the chloroform with soil. The other set of bottles was treated similarly without adding chloroform. The bottles were incubated in the dark for 24 h, and then placed under a fume hood at 40-50°C until all chloroform had evaporated. After removal of even the traces of chloroform, 140 ml of 0.5 M K_2SO_4 was added to both fumigated and unfumigated samples. The bottles were then shaken in an end-to-end shaker for 30 min. The extract from each bottle was filtered through Whatman No.42 filter paper, the optical density at 280 nm immediately taken, and the microbial carbon biomass measured (as per Nunan *et al*. 1998) using aliquots of K_2SO_4 extracts through dichromate digestion. Microbial carbon biomass was calculated after back titration with ferrous ammonium sulphate using the equation:

$$\text{biomass C} = 2.64 \times E_C$$

where E_C = (organic C from fumigated soil) – (organic C from unfumigated soil), expressed as mg kg^{-1} soil. This technique was modified from the usual fumigation method and proved to be more reliable and reproducible.

Alkaline phosphatase activity was assayed in soil suspended in modified universal buffer (pH 11), along with substrate p-nitro phenyl phosphate (Tabatbai & Bremner 1969). After incubation for 1hr at 37 ^{0}C, the enzyme reaction was stopped by addition of 0.5M $CaCl_2$ and 0.5M NaOH. The suspension was filtered through Whatman No 1 filter paper and absorbance measured at 440 nm; enzymatic activity was expressed as µg p-nitro phenol released g^{-1} soil h^{-1}.

Microbial activity was measured using dehydrogenase activity and by FDA hydrolysis using the following techniques. Soil was incubated with triphenyl tetrachloride (3%) for 24 h in the dark. Methanol was added to terminate the enzymatic reaction, the supernatant was filtered, and the absorbance taken at 485 nm (Casida *et al*. 1964). The values were expressed as µg of triphenyl formazone (TPF) released g^{-1} day^{-1}. The FDA (fluorescein diacetate) hydrolysis assay was carried out using soil, potassium phosphate buffer (pH 7.6) and FDA (0.5 mg ml^{-1}). After incubation for 2 h at 37 ^{0}C, the reaction was stopped using acetone. The solution was filtered through Whatman No 1 filter paper and the absorbance of the supernatant taken at 490

nm using a fluorescein standard (Adam & Duncan 2001). The values were represented as µg fluorescein released g^{-1} h^{-1}.

Soil organic carbon was measured using the methodology of Hesse (1971), mixing soil samples, $K_2Cr_2O_7$ and H_2SO_4. The mixture was incubated for 30 min in flasks on asbestos sheets. After incubation, distilled water, orthophosphoric acid and Ferroin indicator were added and titrated with 0.5N ferrous ammonium sulphate solution. The values were expressed as % carbon.

Available phosphorus, and available and total nitrogen, were measured by mixing soil samples with charcoal and 0.5N $NaHCO_3$ and kept under shaking conditions for 30 min. The filtrate was mixed with ammonium molybdate (1.5%) and the absorbance at 660 nm measured after the addition of $SnCl_2$ (Olsen *et al*. 1954). The values were expressed as µg P g^{-1} soil. For nitrogen, the Kjeldahl procedure followed Subbiah & Asija (1956), and the values were expressed as available nitrogen in kg N ha^{-1}.

Soil chlorophyll was assayed using preweighed soil cores (from 0-20 cm depth); acetone: DMSO (1:1) was added at a rate of 4 ml g^{-1} soil. The contents were thoroughly shaken and incubated for 48-96 h in the dark at room temperature. Intermittent shaking every 24 h extracted the chlorophyll completely. Optical density values were taken at 663, 645, 630 and 775 nm, and the *chlorophyll a* concentration determined (Nayak *et al*. 2004).

Acetylene reducing activity (ARA) in soil cores was estimated using gas chromatography of ethylene formed (as an index of nitrogenase activity, and expressed as acetylene reducing activity) (Prasanna *et al*. 2003). Commercially available standard ethylene was utilized for quantification, and vials with an equivalent volume of water served as controls. The ARA values were expressed as nmoles ethylene produced g^{-1} soil. Samples were injected into a preconditioned Nucon Model GLC 5500, housing a two-meter long Porapak R stainless steel column and a flame ionization detector. The column temperature was maintained at 100 °C and injector and detector at 110 °C. A flow rate of 35 ml min^{-1} of N_2 served as the carrier gas. Standard ethylene gas was used for calibration and calculations. All values presented are the means of triplicate measurements.

Growth parameters of the rice were recorded at the time of harvest: grain yield (in g per pot) and the weight of 1000 grains (in g).

Statistical analyses subjected the triplicated data for the various parameters to ANOVA (Analysis of Variance) in accordance with the experimental design (completely randomized design) using MSTAT-C statistical package to quantify and evaluate the sources of variation. The Critical Difference (CD) values were calculated for a significance level of 0.05. Standard deviations are depicted in the graphs as error bars.

Results

The present study was undertaken to evaluate a set of cyanobacterial isolates from the rhizosphere of rice and wheat varieties, in terms of their persistence in soil and roots, and their abilities to promote plant growth and enhance soil fertility in a rice crop grown in pots under greenhouse conditions.

As a prelude to characterizing the activities of the inoculated strains, the cyanobacterial populations in soil samples were estimated (Table 1). At harvest stage, the 'most probable number' values ranged from 0.01–10.2 x 10^4, with the highest values in one of the *Anabaena* inoculates (T11), a two-fold increase compared to other strains, followed by two *Nostoc* inoculates (T3, T2). Fifteen of the 20 inoculated strains persisted in the shallow soil up to harvest of the crop, nine showed visible growth in the rhizosphere, and interestingly, all the enrichment cultures raised using root bits exhibited growth. However, only 12 strains persisted in the roots, and the other samples were dominated by the native *Nostoc* and LPP strains, as observed in uninoculated controls.

Table 1: Cyanobacteria at the harvest stage of the Rice crop.
* = based on microscopic observations; LPP denotes *Lyngbya*, *Phormidium*, *Plectonema;* NA = not applicable (uninoculated controls); MPN = 'most probable number' (see Methods); a full dose of NPK is 120:80:60 NPK kg ha^{-1}; treatments 21-23 are controls; Sources, TN = Tamil Nadu, UP = Uttar Pradesh, IARI = Indian Agricultural Research Institute, Delhi

			Soil at depth 0-20 cms			Rhizosphere			Root		
Treatment	Inoculate	Source	MPN ($\times 10^4$)	Persistence *	Other strains*	Growth	Persistence *	Other strains*	Growth	Persistence *	Other strains*
1	*Anabaena*	Rice, Aduthurai, TN	0.17	+	*Nostoc*, *Aphanocapsa*	+	-	*Nostoc* , LPP	+++	+	*Nostoc*, LPP
2	*Nostoc*	Rice, Aduthurai, TN	2.09	+	-	+	+	*Oscillatoria*, *LPP*	+++	+	*Anabaena*, LPP
3	*Nostoc*	Rice, Lucknow, UP	2.28	+	-	++	+	LPP	+++	+	LPP
4	*Nostoc*	Rice, Faizabad, UP	0.01	-	LPP, *Oscillatoria*, *Anabaena*	-	-	-	++	+	*Calothrix*, *Anabaena*
5	*Nostoc*	Rice, Faizabad, UP	0.92	+	*Gloeocapsa*	-	-	-	+++	+	LPP
6	*Calothrix*	Rice, Faizabad, UP	1.66	+	*Nostoc*, LPP	+	+	*Nostoc*, LPP	+	+	LPP, *Nostoc*
7	*Nostoc*	Rice, Faizabad, UP	0.02	+	-	++	+	LPP	+	-	LPP
8	*Hapalosiphon*	Rice, Faizabad, UP	0.06	-	*Nostoc*	+	+	*Oscillatoria*	+	-	*Lyngbya*
9	*Anabaena*	Rice, Ghagraghat, UP	0.05	+	*Nostoc*	+	-	LPP	+	-	LPP
10	*Nostoc*	Rice, Ghagraghat, UP	0.01	+	-	++	-	LPP	+	-	*Gloeocapsa*
11	*Anabaena*	Rice, Ghagraghat, UP	10.2	+	*Nostoc*	++++	+	LPP	+	+	LPP
12	*Anabaena*	Rice, Ghagraghat, UP	0.05	-	*Nostoc*	+	+	LPP	+	+	*Calothrix*, *Nostoc*
13	*Nostoc*	Rice, Ghagraghat, UP	0.06	-	*Nostoc*	-	-	LPP	+	-	*Nostoc*,
14	*Nostoc*	Rice, Ghagraghat, UP	0.40	+	-	+	-	*Oscillatoria*	++	+	*Anabaena*
15	*Anabaena*	Rice, Kanpur , UP	0.17	+	*Nostoc*	-	-	-	+	-	*Oscillatoria*, LPP
16	Cyanobiont from *Azolla* sp	IARI, Delhi	0.09	+	*Nostoc*	+	+	*Anabaena*	+	+	LPP
17	Cyanobiont from *Azolla* sp	IARI, Delhi	0.12	+	*Nostoc*	+	-	*Oscillatoria*	+	+	LPP
18	*Calothrix*	Wheat , IARI, Delhi	0.19	-	*Nostoc*	+	-	*Nostoc*	+	-	*Nostoc*, LPP
19	*Hapalosiphon*	Wheat , IARI, Delhi	0.79	+	*Nostoc*	+	-	*Calothrix*, LPP, *Nostoc*	+	-	*Calothrix*
20	*Nostoc*	Wheat , IARI, Delhi	0.68	+	LPP	+	+	*Calothrix*, LPP	+	+	*Calothrix*, LPP
21	(1/3 NPK)		0.05	NA	*Nostoc*	-	NA	*Desmids*	+	NA	LPP
22	(2/3 NPK)		0.40	NA	*Nostoc*	+	NA	*Oscillatoria*	++	NA	*Nostoc*, LPP
23	(NPK)		0.33	NA	*Nostoc*, LPP	+	NA	LPP	+	NA	*Nostoc*, *Gloeocapsa*, *Anabaena*

Treatments involving inoculation with strains T1 and T10 showed the highest values of FDA hydrolysis, which were more than values recorded with the control full dose of NPK

fertilizers (Table 2). The highest dehydrogenase activity was recorded on inoculation with strains T1 and 11, which were also significantly higher than those recorded in the control treatments (Fig. 1a). Inoculation with strains T1, 7, 10 and 11 stimulated microbial activity, as evidenced by either enhanced dehydrogenase or FDA activity (Table 2). Inoculation with strain T7 also resulted in the highest alkaline phosphatase activity, followed by treatments T8 and T6 (Table 2), both of which exhibited significantly higher values than even the control full dose NPK treatment (T23).

Table 2: Influence of cyanobacterial inoculation on selected soil microbiological parameters.

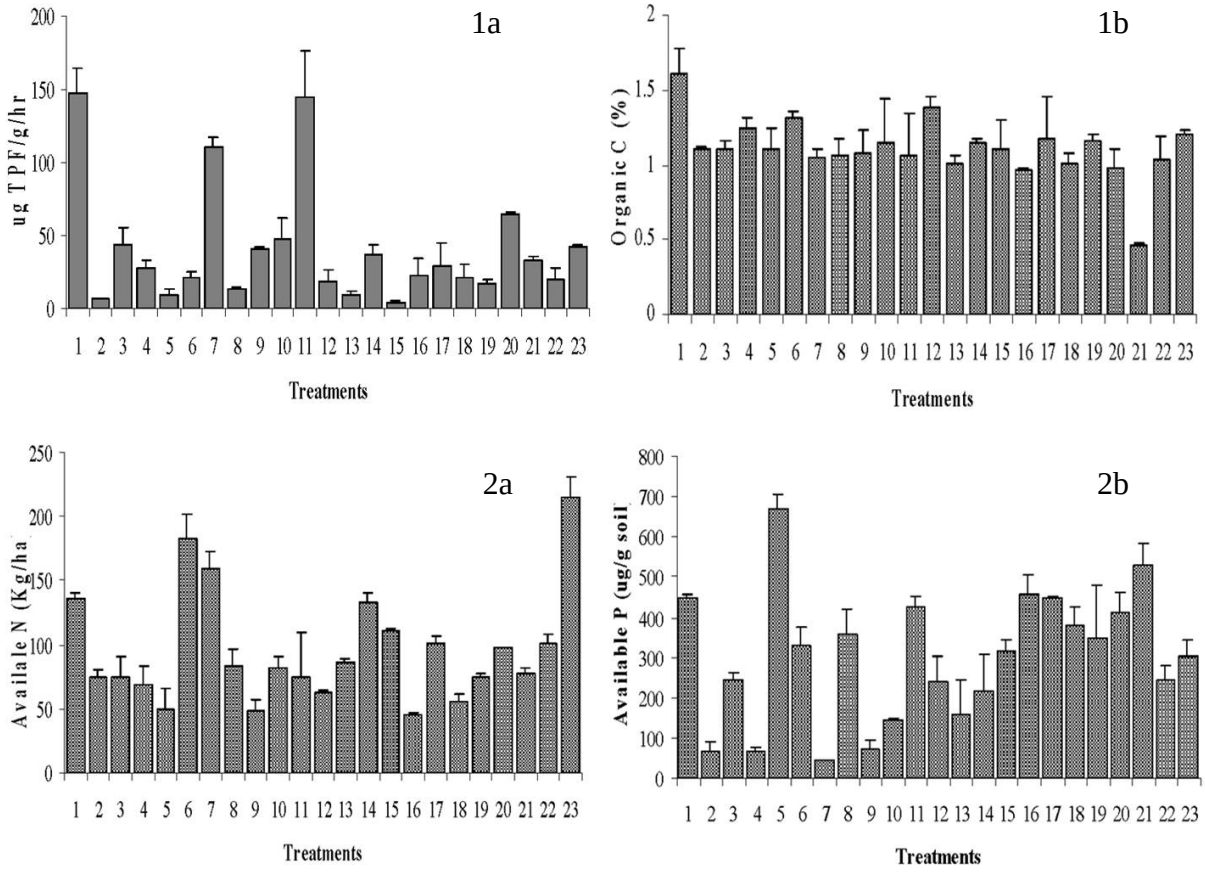
Superscripts denote the ranking of the mean values of each column, based on Duncan's Multiple Range test. C.D. = Critical Difference for the 5% level of significance.

pnp = p-nitro phenol; C_{mic} = microbial carbon; C_{org} = organic carbon

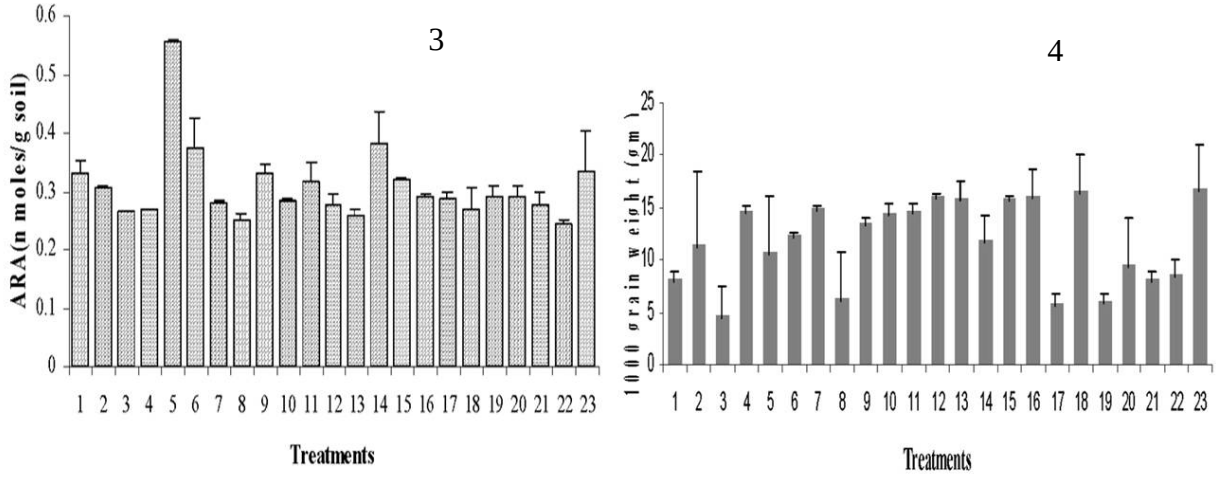
Treatment	Alkaline phosphatase (µg pnp g^{-1} hr^{-1})	FDA hydrolysis (µg fluorescein g^{-1} hr^{-1})	Microbial biomass (µg g^{-1} soil)	Percentage C_{mic} / C_{org}	Chlorophyll (mg chl g^{-1} soil)	Grain yield (g per pot)
T1	79.93 ^{6}	0.933 ^{1}	313.1 ^{10}	1.95	2.287 ^{4}	2.60 ^{12}
T2	78.89 ^{6}	0.370 ^{5}	349.1 ^{9}	3.17	3.197 ^{2}	4.57 ^{9}
T3	29.32 ^{14}	0.227 ^{8}	372.3 ^{8}	3.38	1.663 ^{6}	0.60 ^{18}
T4	67.12 ^{7}	0.293 ^{6}	361.7 ^{8}	2.91	1.720 ^{6}	5.67 ^{5}
T5	37.53 ^{13}	0.340 ^{6}	525.7 ^{3}	4.77	2.587 ^{3}	6.10 ^{4}
T6	88.23 ^{3}	0.393 ^{4}	507.1 ^{4}	3.84	2.243 ^{4}	5.15 ^{6}
T7	115.50 ^{1}	0.420 ^{3}	104.1 ^{15}	0.99	1.053 ^{10}	2.29 ^{13}
T8	100.00 ^{2}	0.340 ^{6}	353.6 ^{9}	0.85	1.290 ^{8}	3.14 ^{10}
T9	64.43 ^{8}	0.320 ^{6}	169.4 ^{13}	1.58	0.737 ^{12}	4.99 ^{7}
T10	67.56 ^{7}	0.837 ^{1}	601.3 ^{2}	5.22	1.237 ^{9}	2.56 ^{12}
T11	81.96 ^{5}	0.293 ^{6}	117.1 ^{14}	1.10	0.847 ^{12}	5.88 ^{5}
T12	49.49 ^{11}	0.203 ^{9}	852.7 ^{1}	6.17	0.507 ^{14}	7.14 ^{3}
T13	8.55 ^{15}	0.200 ^{9}	66.9 ^{16}	0.66	1.677 ^{6}	9.10 ^{1}
T14	79.58 ^{6}	0.177 ^{10}	18.0 ^{17}	0.15	2.210 ^{4}	4.90 ^{8}
T15	71.18 ^{7}	0.103 ^{11}	448.3 ^{5}	4.03	0.577 ^{13}	2.30 ^{14}
T16	60.65 ^{9}	0.200 ^{9}	442.5 ^{6}	4.60	1.741 ^{5}	2.43 ^{13}
T17	33.79 ^{14}	0.260 ^{7}	232.4 ^{12}	1.97	4.597 ^{1}	1.40 ^{16}
T18	39.31 ^{12}	0.220 ^{8}	17.2 ^{17}	0.17	1.363 ^{7}	8.96 ^{2}
T19	60.48 ^{9}	0.313 ^{6}	335.7 ^{10}	2.87	0.987 ^{11}	0.89 ^{17}
T20	57.25 ^{10}	0.427 ^{3}	420.6 ^{7}	0.43	0.433 ^{15}	1.85 ^{15}
T21	36.19 ^{13}	0.287 ^{6}	542.1 ^{3}	1.17	0.580 ^{13}	2.75 ^{11}
T22	39.82 ^{12}	0.253 ^{7}	119.6 ^{14}	1.15	1.357 ^{7}	3.16 ^{10}
T23	85.93 ^{4}	0.593 ^{2}	275.6 ^{11}	2.28	1.230 ^{9}	7.40 ^{3}
C.D.(p=0.05)	19.37	0.143	57.5		0.612	2.39

Enhancement was observed in soil organic carbon in all the treatments compared with control T21, and strains T1, 12 and 6 recorded significantly higher values (Figure 1b) than all control treatments. Microbial carbon biomass was significantly higher in pots inoculated with strains T12 and 10 (Table 2), and was positively correlated with organic C (r^2 = 0.48). Interestingly, photosynthetic biomass measured as soil chlorophyll showed significant enhancement in nine of the strains over control treatments (Table 2), and also over the applied (initial) inoculum level of 5 µg ml^{-1} chlorophyll. The highest values of microbial biomass and organic carbon ratio (C_{mic} / C_{org}), (Table 2) were recorded in T12, followed by T10.

Figures 1 & 2: Influence of cyanobacterial inoculation (treatments 1–23 as in Table 1) on various soil parameters: control treatments are T21-23
1a = soil dehydrogenase activity (µg TPF g^{-1} soil d^{-1}); **1b** = organic carbon (%).
2a = available nitrogen (kg ha-1); **2b** = available phosphorus (µg g^{-1} soil).



Figures 3 & 4. Influence of cyanobacterial inoculation (treatments 1–23 as in Table 1) on various soil parameters
3 = acetylene reducing activity (nmol g^{-1} soil, an index of nitrogen fixation).
4 = weight of 1000 grains of rice (g)



Treatment involving inoculation with strain T6 recorded the highest values for available nitrogen, followed by T7, both of which were lower than the control treatment

involving the full dose of chemical fertilizers. Available phosphorus was much higher in T5 as compared to all the controls (Figures 2 a, b). Soil acetylene reducing activity (an index of nitrogen fixation) was also much higher in pots receiving inoculation with strains T5 and 14, as compared to controls (Figure 3). The inoculated treatments T15, 18, 13, 12 and 11 recorded significantly higher yields than control T23 (the full dose of NPK). All the strains except T3, 8, 16, 17, and 19 recorded higher or statistically equal values to controls T21 and T22 (1/3 and 2/3N +P + K treatments) (Table 2). In terms of grain weight (Fig. 4), the highest values were recorded in the treatment involving the full dose of NPK, but inoculation with strains T18, 16 and 15 were statistically equivalent.

Discussion

Rhizobacteria that promote plant growth are defined as a type of biofertilizer which directly helps to provide nutrients to the host plant, or indirectly influences positively root growth and morphology, or aids other beneficial symbiotic relationships. Among these, facultative root interactions at the rhizospheric level have great significance in ecological and sustainable resource management in agriculture, which include interactions aiding in nutrient exchange, mobilization of exudates/enzymes and modification of root structure (Vessey 2003, Bohme & Bohme 2006). The potential of cyanobacteria to promote plant growth has been recently demonstrated in wheat crop under glasshouse and Phytotron conditions (Karthikeyan *et al.* 2007), but no published information exists on their value in rice except in laboratory investigations. Cyanobacteria characteristically liberate substantial quantities of extra-cellular nitrogenous compounds into the medium irrespective of whether they are growing on elemental nitrogen or on combined nitrogen, and the production of combined nitrogen in soluble form by nitrogen-fixing cyanobacteria has been recorded by several researchers (Singh 1961, Venkataraman 1972, Vorontsova *et al*. 1988), with estimated amounts of N_2 fixed as a result of cyanobacterial inoculation in the range of 20-30 kg ha^{-1}. There is conclusive evidence that nitrogen fixed by cyanobacteria is made available to rice as well as other plant or microbial life (Mandal *et al*. 1998, Nayak *et al.* 2004).

Among the set of cyanobacterial strains tested, seven (2, 3, 6, 11, 12, 16 and 20) persisted in the rhizosphere soil and root samples, and exhibited good growth in the respective enrichment cultures. Such strains with superior establishing traits may prove to be better competitors when used as inoculants. Competition among symbiotic cyanobacteria strains forming artificial associations has been studied by Nilsson *et al*. (2005), who showed that rice roots attract *Nostoc* strain 9104, and the attraction varies depending on the temperature and whether the plant had been grown with or without nitrogen. The pervasiveness of cyanobacteria in the rhizosphere and their diversity (Prasanna *et al.* 2009) indicates their potential as inoculants.

Microorganisms are the logical choice for supplying most of the soil's enzyme activity because of their large biomass, high metabolic activity and relatively larger amounts of extra cellular enzymes than plants or animals. The activity of enzymes can be used as an index of the fertility status of soil as they essentially integrate the effects of climate, crops and soil amendments and edaphic properties.

The enhancement in soil fertility is known to be responsible for the maintenance of sustainable yields in rice, especially as a result of cyanobacterial inoculation. Phosphorus is known to be second only to nitrogen in mineral nutrients, most commonly limiting the growth of crop plants, mainly because of the low available amounts, despite large reserves. Plant growth promoting rhizobacteria are also known to increase the availability of nutrients in the rhizosphere, which may be through solubilisation of unavailable forms of nutrients and/or siderophore production and facilitating transport of nutrients (Arshad & Frankenberger 1998, Vessey 2003). Alkaline phosphatases are of great importance in soil organic phosphate

mineralization and plant nutrition (Klose & Tabatbai 2002). A significant enhancement in alkaline phosphatase activity over control was recorded in our investigation, illustrating the significance of cyanobacterial inoculants in P mineralization.

Soil microbial biomass is fundamental to maintaining soil functions as it represents the main source of soil enzymes that regulate transformation processes of elements in soils. It also controls the build-up and breakdown of organic matter, the decomposition of organic residues, and serves as early indicator of changes in soil management, heavy metal contamination and fertilizer practices. Microbial biomass and the organic carbon ratio (C_{mic} / C_{org}), expressed as a percentage, ranged from 0.15 to 6.17 in our study and signifies the important contribution of cyanobacterial inoculation. This ratio is known to be an indicator of conversion efficiency of organic C into microbial C, and of losses of soil C during decomposition, besides being useful as a soil-quality indicator. Although Jenkinson & Ladd (1981) proposed a ratio of 2.2% for C_{mic}/C_{org} as an equilibrium threshold for cultivated soils, Anderson & Domsch (1989) emphasized that this ratio varies widely (from 0.27 to 7.0%) across different soil management systems, sampling times and analytical methods. A build-up of organic matter due to algal inoculation has been demonstrated by many workers (Aiyer *et al*. 1971, Karthikeyan *et al*. 2007). An increase of 0.03% in soil organic carbon content equivalent to 670 kg ha^{-1} was reported due to enrichment of native algal flora alone under laboratory conditions over a six-month period (Kaushik 2004), whereas inoculation of halo-tolerant cyanobacterial strains to sodic soil resulted in an addition of 5.3-7.6 t carbon ha^{-1} per cropping season. In our study, the cyanobacterial strains exhibiting high C_{mic}/C_{org} ratio (i.e. T12, 10 and 5) could prove beneficial for soil carbon enrichment.

An increase in the available nitrogen in rice soil due to the growth of N-fixing cyanobacteria was reported a long time ago (Singh 1961). Chemical fixation of P is a common problem in all types of soils (Gaur 1990), and hence a linear relationship between increasing levels of N and available P was not observed in the soil samples at harvest, also explicable because of differences in the abundance and diversity of the native flora enriched as a result of the N applications. However, our observations clearly indicate a definite enrichment in C, N and P through inoculation of specific cyanobacterial strains (especially T7 and T1).

This study provides evidence regarding the significance of cyanobacterial biomass, not only as agents of carbon sequestration in soils, but also as significant contributors to enhanced microbial activity and plant-growth promotion, thereby emphasizing their significance in the sustainable management of the rice ecosystem. Further studies are being undertaken for field scale evaluation, and in the use of tagged inoculants to monitor populations in soil, which may help to further strengthen our observations.

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الملخص العربى

حركة الريزوسفير للسيانوبكتريا ودورها فى تأهيل النمو فى محصول الأرز

رادها براسانا – لاتا ناين – راديكا أنشا – شاداف سريكريشينا – مومينكا شوشى – براهما كواشيك
قسم الميكروبيولوجى – معهد البحوث الزراعية الهندى – نيودلهى – الهند

إن عملية تثبيت النتروجين فى التربة تتم عن طريق السيانوبكتريا والتى تنتشر فى الحقول الزراعية وفيرة المياه مما يؤدى إلى زيادة كمية النتروجين مما يكون له تأثير على النظم البيئية. إن تجهيز تلك البكتيريا ومعرفة دورها فى نمو النبات ونشاطها الميكروبى داخل التربة من الموضوعات قليلة الدراسة.

ولذا فقد تمت هذه الدراسة تحت ظروف الصوبا الزجاجية من خلال حقن جذور نبات الآرز بأنواع مختلفة من السيانوبكتريا المختارة والتى تتمثل فى حوالى 20 نوع والتى تم عزلها من ريزومات أنواع مختلفة من الآرز والقمح.

أثبتت الدراسة أن أنواع عديدة من البكتريا قد استقرت فى التربة وداخل جذور النبات وظلت موجودة حتى موسم الحصاد، مما أدى إلى زيادة كمية الكربون والنتروجين وباقى العناصر الميكروبيولوجية الآخرى وذلك بصورة معنوية مما أدى إلى زيادة الإنتاج النهائى ووزن الحبوب. ولذا يمكن القول بأن هذه الدراسة أوضحت ان هناك بعض الانواع من البكتريا التى يمكن حقنها فى التربة مما يؤدى إلى زيادة خصوبة التربة والإنتاج النهائى للمحاصيل.