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ISOLATION, ENUMERATION AND IDENTIFICATION OF DIAZOTROPH STRAINS FROM ACIDIC RAINFED LOWLYING RICE AGRO-ECOSYSTEMS OF SOUTH ASSAM (BARAK VALLEY), INDIA

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In this study, strains of indigenous diazotroph were isolated from rhizosphere soils of rice grown in acidic low lying rainfed ecosystems on N-free Burk's and malate-sucrose media by dilution plating method. Morphological and biochemical study as per Bergey's manual of determinative bacteriology revealed that the strains belonged to nine species namely, *Azotobacter chroococcum, Azospirillum amazonense, Beijerinckia indica, Burkholderia caribensis, Gluconacetobacter liquefaciens, Acinetobacter johnsonii, Derxia gummosa, Bacillus polymyxa* and *Pseudomonas fluorescence.* The cfu count of these strains varied significantly in response to cropping season. *A. chroococcum, A. amazonense, B. caribensis, G. liquefaciens* and *B. polymyxa* have higher rhizosphere population in autumn (sali/pre-kharif) season. *B. indica, A. johnsonii, D. gummosa* and *P. fluorescence* have higher rhizosphere count in summer (ahu/rabi) season. The present study also revealed that *A. chroococcum, A. amazonense, B. caribensis* and *G. liquefaciens* showed increase in viable cell count with concomitant increase in soil pH but *B. indica* and *A. johnsonii* count decreases with increasing soil pH. *D. gummosa, B. polymyxa* and *P. fluorescence* count was least effected by variation in soil pH. The nitrogenase activity of the diazotroph strains ranged from 114.80 to 413.15 nM C_2H_2 hr^{-1} mg^{-1} protein. Among the identified diazotroph strains, *A. chroococcum, A. amazonense, B. caribensis, B. indica, G. liquefaciens* and *A. johnsonii* have showed higher nitrogen fixing potential which may be used as biofertilizers for intensive rice cropping in acidic rainfed tropical lowlands.

Keywords: Biofertilizer; cfu; Diazotroph; Nitrogenase activity; Rainfed lowland.

Introduction

Rice is the major staple food of the people residing in tropical countries of Asia. Rice requires about 1 kg of chemical N fertilizer to produce 15 - 20 kg of grain[1]. Low input efficiency of N fertilizer in tropical rainfed rice agro-ecosystems, decline in rice yield under continuous cropping, nitrate pollution, acidification of soils and emission of greenhouse gases such as ammonia and nitrous oxide, are some economic and environmental factors associated with the use of chemical N fertilizers[2]. In addition, rundown of N supply capacity of agricultural soils, lack of purchase power of impoverished farmers, gap between the researchers and farmers in N management of crops and soils for maximum production efficiency, have confounded the situation still further[3]. Chemical N fertilizers not only deplete non-renewable resources, but also pose human and environmental hazards. These concerns have renewed public interest in exploiting alternative or supplementary N sources to encourage sustainable rice farming.

In the case of nitrogen inputs into rice production, it may be the judicious use of N fertilizers or N derived from atmospheric N via biological nitrogen fixation (BNF). The microbes capable of BNF are termed as 'diazotrophs', as these convert atmospheric di-nitrogen (N_2) to ammonia (NH_3) by electron reduction and protonation of gaseous N_2. The nitrogenase enzyme complex in diazotrophs is mainly responsible for such N fixation activity. These diazotrophs comprises diverse N-fixing microbes, envisaging aerobes (e.g. *Azotobacter, Beijerinckia, Derxia,* etc.), facultative anaerobes (e.g. *Clostridium, Pseudomonas, Rhizobium* etc.), heterotrophs (e.g. *Klebsiella, Enterobacter,* etc.) and phototrophs (e.g. *Anabaena, Azospirillum, Nostock,* etc.). The free-living or associative N_2-fixing diazotrophs with the roots of cereals and grasses contributes from 10 to 80 kg N /hectare/

cropping season[4]. In addition to N contribution these bacteria also improve the nutrient transformation and contribute to plant growth promoting effects. For the last one-decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical N fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere[5]. Keeping the above facts in mind, the present investigation has been undertaken to isolate, enumerate and identify the indigenous diazotroph strains from the rhizosphere soil of rice grown in acidic rainfed lowlands of South Assam, India and screen their nitrogen fixing potential which might help in reducing the use of costly and hazardous chemical N fertilizers in the cultivation of rice in tropical countries.

Material and Methods

To isolate diazotroph strains from rice rhizosphere soil, samples were collected randomly from 19 locations of Cachar district, 14 locations of Karimganj district and 7 locations of Hailakandi district from the rice growing fields during July' 2007 to May'2009 representing four cropping seasons; sali(autumn)'2007, ahu(summer)' 2008, sali(autumn)' 2008 and ahu(summer)' 2009. Three, parallel sampling lines were marked out at known distances (depending on the size of the selected rice growing field) from each other in each location. The first line was placed randomly and the others parallel to this line. Each line included four sampling areas ($1m^2$) placed at regular distances from each other. Five rectangular soil cores (5 × 3.5 cm^2, 0-30 cm deep) were taken from each sampling area involving rhizospheric zone of growing rice crop. The samples taken from the same line were combined and each pooled soil sample, henceforward consisting of 20 cores. The soil samples were placed in sterilized plastic bags, transferred to the laboratory within one day and stored at 4°C prior to isolation. Isolation and purification of diazotroph strains were carried out in Burk's N-free medium[6] and N-free malate-sucrose medium (NFMM) modified from Dobereiner[7]. Isolation of diazotroph strains from all three pooled soil samples of each location (n=3) was carried out by the dilution plate count method[8, 9]. Colonies growing on the dishes were counted after incubation. Data from each of the three replicates were averaged for a soil sample and expressed as CFU (colony forming units) per gram of oven dried soil. Different types of single, well-separated colonies growing on the plate were picked and transferred into agar slants with respective solid N free media. Pure culture for each diazotroph strain was obtained by streaking agar plates of respective medium with inoculums from slant tubes. Identification of diazotroph isolates was done by (a) micromorphology (b) cultural, biochemical and physiological study following Bergey's manual of determinative bacteriology[10]. Acetylene reduction assay (ARA) was used for measuring the rate of N_2-fixation of the diazotroph strains[11]. Values were expressed as nmoles C_2H_4 h^{-1} mg^{-1} protein. The occurrence of diazotroph strains at different soil pH was analyzed to determine the effect of soil reaction on the growth of diazotroph strains. The pH of the pooled soil samples was measured following standard method of soil analysis[12]. ANOVA test ($p<0.05$) was performed to compare the population of diazotrophs in different cropping seasons as well as to compare the population of different diazotrophs among themselves utilizing Dunkun's Multiple Range test[13]. Summary statistics were used to obtain the mean and standard error[14]. The least significant difference and correlation analysis were carried out following the method of Misra and Misra[15].

Results and Discussion

Altogether, nine strains of indigenous diazotroph were isolated from the acidic rice rhizosphere soils of South Assam, India viz., *A. chroococcum, A. amazonense, B. indica, B. caribensis, G. liquefaciens, A. johnsonii, D. gummosa, B. polymyxa* and *P. fluorescence.* The data in Table 1 shows the population of diazotroph strains in rice rhizosphere soils of the three districts of South Assam in different cropping seasons (autumn and summer). In Cachar district, highest cell count of *A. chroococcum* (79 ± 1.88 × 10^6), *A. amazonense* (67 ± 6.67 × 10^6), *B. caribensis* (51 ± 4.32 × 10^6), *G. liquefaciens* (32 ± 2.3 × 10^6), *B. polymyxa* (36 ± 2.99 × 10^6) and *P. fluorescence* (30± 2.42 ×10^6) were reported in sali (autumn) season. *B. indica* (52 ± 5.27 × 10^6), *D. gummosa* (50 ± 4.14 ×10^6) and *A. johnsonii* (34 ± 0.85 × 10^6) showed higher cell count in ahu (summer) season in the rice fields of Cachar district. The cell count of *A. chroococcum* (76 ± 5.22 × 10^6), *A. amazonense* (70 ± 2.34 × 10^6), *B. caribensis* (52 ± 4.42 × 10^6), *G. liquefaciens* (37 ± 1.26 × 10^6) and *B. polymyxa* (32 ± 3.16 × 10^6) were higher in sali (autumn) season in the rice agro-ecosystems of Karimganj district. The population of *B. indica* (46 ± 2.97 × 10^6), *D. gummosa* (42 ± 1.87 × 10^6), *P. fluorescence* (42 ± 0.00 ×10^6) and *A. johnsonii* (28 ± 2.2 ×10^6) were higher in ahu (summer) season in the rice agro-ecosystem soils of Karimganj district. In case of Hailakandi district, *A. chroococcum* (80 ± 3.90 × 10^6), *A. amazonense* (71 ± 5.61 × 10^6), *B. caribensis* (57 ± 4.5 ×10^6), *G. liquefaciens* (37 ± 1.42 × 10^6) and *P. fluorescence* (18 ± 1.42 × 10^6) showed higher count in sali (autumn) season. *D. gummosa* (41 ± 1.32 × 10^6), *B. indica* (58 ± 3.23 × 10^6) and *A. johnsonii* (32 ±

2.82×10^6), shows higher number in ahu (summer) season in the rice fields of Hailakandi district. On an average the population of diazotrophs was higher in sali (autumn) season than that in ahu (summer) season. The population of diazotrophs differed significantly not only between two cropping seasons but between the strains also (Table 2). The seasonal variation of N_2-fixing bacterial population was highest in the rice fields of Karimganj district. The maximum difference in the population of diazotroph strains among themselves was envisaged in the rice fields of Hailakandi district. On an average, *A. chroococcum* population was highest followed by *A. amazonense* and *B. caribensis* which are the dominant diazotroph strains in the rice rhizosphere soils of South Assam. The cell count of *G. liquefaciens, B. indica* and *D. gummosa* was in the medium range in the rice fields and contributes to the indigenous diazotroph diversity of South Assam. The population of *B. polymyxa, P. fluorescence* and *A. johnsonii* was lower in comparison to the other strains signifying their less potential to supply nitrogen needs of rice.

The climatic condition of South Assam favours rice cultivation in tropical lowlands. The zone is characterized by heavy annual rainfall over 2500 mm, medium temperature range (15-35°C) and high relative humidity (>75%). Maximum rainfall occurs between May to August lowering the soil pH to its minimum.

The pH of pooled soil samples of three districts ranged from 4.80 to 6.50. The soil pH decreased with increase in rainfall in the summer months effecting the population of diazotrophs in the flooded rice fields of Barak Valley. The acidity of soil decreased in the middle part of autumn season and consequently the CFU count of diazotrophs increased in the rice fields. To find the effect of soil pH on the population of diazotrophs, the number of viable cells per gram of soil of the isolated strains was correlated with the pH values of pooled soil samples. Table 3 showed that *A. chroococcum* population is positively correlated with the soil pH which supported its higher CFU count in autumn season at increased soil pH. *A. amazonense, B. caribensis* and *G. liquefaciens* also showed positive correlation with soil pH confirming occurrence of higher number of viable cells per gram of soil in autumn season in the rice agro-ecosystems of Barak Valley. The population of *B. indica* and *A. johnsonii* showed negative correlation with soil pH indicating occurrence of lower number of viable cells per gram of soil at increased soil pH in autumn season. *D. gummosa* and *P. fluorescence* count was higher in summer and early autumn season and showed partial negative correlation to soil pH. *B. polymyxa* count was more in early autumn season accounting partial positive correlation towards soil pH.

A. chroococcum strain was confirmed on the basis of following characteristics: smooth opaque convex circular gummy colony with undulated margin, cells ovoid rods or cocci, size 2 ×1.5 µm, older cells produce brown to black pigments, presence of thick walled cysts, presence of flagella, cells occur singly or in pairs or in chains or in irregular clumps, gram-negative, catalase positive, aerobic and produce extracellular gum.

A. amazonense strain was identified on the basis of characteristics: white dense pin point colony with irregular margin, subsurface white pellicle in semisolid medium, cells vibroid rods, size 2 × 0.5 µm, absence of cysts, gram negative, catalase variable, microaerophillic, presence of flagella and polysaccharide crystals in the cell.

B. indica strain was confirmed on the basis of characteristics: smooth irregular folded and raised colonies, produces tenacious and elastic slime, cells curved rods, size 2.0 × 1.0 µm, occur singly, presence of cysts, gram negative, absence of flagella, catalase positive, aerobic and presence of intracellular polysaccharide crystals.

B. caribensis strain has been identified as per following characteristics: round, convex, smooth, opaque orange colony, cell rod shaped and occur singly, size 2.0 × 1.2 µm, gram negative, cyst absent, extracellular granule absent, catalase positive, nonsporulating, motile, single flagellum, aeobic and nonfluorescent.

G. liquefaciens strain has following characters: irregular, smooth, flat, mucoid yellowish colony, gram negative, motile, flagella present, rod shaped cells with rounded ends, occur in chains, size 1.6 × 0.5 µm, catalase positive, aerobic, oxidase negative and nonfluorescent.

A. johnsonii strain was confirmed on the basis of its characteristics: circular, convex, smooth mucoid, non-pigmented colonies with entire margin, cells rod and occur in chains, size 1.7 × 0.6 µm, gram negative, catalase positive, aerobic, non-sporulating, non-motile, and presence of fimbriae.

D. gummosa strain showed following characters: massive opaque highly raised slimy colonies with wrinkled surface, older colonies become dark brown, cells rod shaped and occur in short chains, size 2.5 × 1.0 µm, gram negative, absence of cysts, produces extracellular slime, catalase positive, aerobic and presence of flagella.

Cells of *B. polymyxa* were confirmed from the following characters: circular white colony beneath the agar surface, cells rod shaped, size 2.5 × 2 µm, gram positive, catalase negative, absence of cysts, flagella,

Table 1. Enumeration of diazotroph strains in rice rhizosphere soils of South Assam in different cropping seasons.

District	Strain code	Strain identification	*CFU / g of dry soil (× 10^6) Sali season'07	Ahu season'08	Sali season'08	Ahu season'09
Cachar	SDSA-I12/2	*A. chroococcum*	63 ± 2.40	38 ± 2.09	79 ± 1.88	39 ± 2.46
	SDSA-I14/1	*A. amazonense*	56 ± 3.04	31 ± 2.40	67 ± 6.67	35 ± 2.02
	SDSA-I30/2	*B. indica*	15 ± 1.32	41 ± 3.65	8 ± 1.20	52 ± 5.27
	SDSA-I10/1	*B. caribensis*	43 ± 1.75	24 ± 1.56	51 ± 4.32	30 ± 3.1
	SDSA-I28/1	*G. liquefaciens*	32 ± 2.3	0	26 ± 2.3	0
	SDSA-I19/1	*A. johnsonii*	0	34 ± 0.85	12 ± 1.12	27 ± 2.16
	SDSA-I28/2	*D. gummosa*	12 ± 0.28	37± 0.78	21 ± 0.95	50 ± 4.14
	SDSA-I22/1	*B. polymyxa*	26 ± 2.78	30 ± 3.90	36 ± 2.99	28 ± 1.81
	SDSA-I16/1	*P. fluorescence*	0	18 ± 1.20	30± 2.42	29 ± 2.20
Karimganj	SDSA-I12/2	*A. chroococcum*	59 ± 2.26	38 ± 2.43	76 ± 5.22	37 ± 2.20
	SDSA-I14/1	*A. amazonense*	67 ± 2.96	21 ± 2.84	70 ± 2.34	26 ± 2.02
	SDSA-I30/2	*B. indica*	0	28 ± 1.47	18± 1.25	46 ± 2.97
	SDSA-I10/1	*B. caribensis*	0	28 ± 3.1	52 ± 4.42	21 ± 1.32
	SDSA-I28/1	*G. liquefaciens*	37 ± 1.26	12 ± 0.90	31 ± 2.8	7 ± 0.74
	SDSA-I19/1	*A. johnsonii*	5 ± 0.40	28 ± 2.2	0	20 ± 1.62
	SDSA-I28/2	*D. gummosa*	18 ± 0.80	36 ± 3.00	42 ± 1.87	0
	SDSA-I22/1	*B. polymyxa*	23 ± 3.29	27 ± 3.21	32 ± 3.16	12 ± 0.00
	SDSA-I16/1	*P. fluorescence*	22 ± 1.12	42 ± 0.00	28 ± 2.2	11 ± 0.87
Hailakandi	SDSA-I12/2	*A. chroococcum*	80 ± 3.90	48 ± 2.00	73 ± 2.81	43 ± 4.80
	SDSA-I14/1	*A. amazonense*	68 ± 3.10	35 ± 3.04	71 ± 5.61	38 ± 1.98
	SDSA-I30/2	*B. indica*	21 ± 2.12	44 ± 4.87	15 ± 0.00	58 ± 3.23
	SDSA-I10/1	*B. caribensis*	47 ± 2.82	17 ± 1.25	57 ± 4.5	24 ± 2.1
	SDSA-I28/1	*G. liquefaciens*	37 ± 1.42	10 ± 2.02	31 ± 3.12	0
	SDSA-I19/1	*A. johnsonii*	0	32 ± 2.82	17 ± 1.2	25 ± 1.85
	SDSA-I28/2	*D. gummosa*	26 ± 1.71	41 ± 1.32	19 ± 0.90	32 ± 1.90
	SDSA-I22/1	*B. polymyxa*	23 ± 0.85	28 ± 2.81	26 ± 2.35	0
	SDSA-I16/1	*P. fluorescence*	18 ± 1.42	0	12± 1.12	22 ± 1.12

± SeM, * CFU/g of dry soil is the average of all the locations in each district which was again average of the three pooled soil samples of each location in a district.

intracellular polysaccharide crystals and extracellular gum.

P. fluorescence showed the following characters: semitransparent raised irregular colony with wrinkled surface, cells rod shaped, size 2.5 × 1.5µm, gram negative, catalase positive, absence of cysts, flagella present, absence of gum and intracellular polysaccharide crystals.

The pure cultures of the isolated strains of N_2-fixing bacteria were tested for the ability to fix atmospheric N_2 by acetylene reduction technique. The more the activity of nitrogenase enzyme the greater is the ability of atmospheric N_2-fixation. The maximum nitrogenase activity was observed in *A. chroococcum* culture (413.15 nM C_2H_4 $hr^{-1}mg^{-1}$ protein). *B. caribensis, A. amazonense, G. liquefaciens, B. indica* and *A. johnsonii* cultures showed good N_2-fixation ability and ARA activity was more than 300.0 nM C_2H_4 $hr^{-1}mg^{-1}$ protein. *B. polymyxa, D. gummosa* and *P. fluorescence* cultures has less activity of nitrogenase enzyme indicating poor nitrogen fixing ability (Table 4).

Nine strains of N_2-fixing heterotrophic bacteria viz., *A. chroococcum, A. amazonense, B. indica, B. caribensis, G. liquefaciens, A. johnsonii, D. gummosa, B. polymyxa* and *P. fluorescence* were isolated from the rhizosphere region of cultivated rice varieties grown in the tropical rainfed lowlands of South Assam, India. This

Table 2. Analysis of variance of the data on quantitative enumeration of diazotrophs in rice rhizosphere soils of South Assam.

District	Source of variation	Degree of freedom	Calculated F	Tabulated F	Probability level	Significance level
Cachar	Between Seasons	$n_1 = 3$ $n_2 = 24$	5.61	4.7	0.01	**
	Between diazotrophs	$n_1 = 8$ $n_2 = 24$	4.28	3.4	0.01	**
Karimgan	Between seasons	$n_1 = 3$ $n_2 = 24$	5.12	4.7	0.01	**
	Between diazotrophs	$n_1 = 8$ $n_2 = 24$	2.79	2.4	0.05	*
Hailakandi	Between Seasons	$n_1 = 3$ $n_2 = 24$	6.68	4.7	0.01	**
	Between diazotrophs	$n_1 = 8$ $n_2 = 24$	6.94	5.0	0.001	***

***-Significant at 5%, 1% and 0.1% probability level. **-Significant at 5% and 1% probability level. *-Significant at1% probability level.

Table 3. Correlation analysis of the CFU count of diazotroph strains and soil pH.

Diazotrophs	Calculated value of r at 11 degree of freedom	Significance level	Type of correlation	Regression equation
A. chroococcum	0.970	0.1%	Perfect +ve	$Y_x = -101.21 + 27.79x$
A. amazonense	0.966	0.1%	Perfect +ve	$Y_x = -124.38 + 30.59x$
B. indica	-0.759	0.1%	Perfect -ve	$Y_x = 182.67 - 27.83x$
B. caribensis	0.968	0.1%	Perfect +ve	$Y_x = -120.83 + 27.15x$
G. liquefaciens	0.951	0.1%	Perfect +ve	$Y_x = -114.37 + 23.49x$
A. johnsonii	-0.936	0.1%	Perfect -ve	$Y_x = 128.67 - 19.79x$
D. gummosa	-0.092	5%	Partial -ve	$Y_x = 31.37 - 2.57x$
B. polymyxa	0.229	5%	Partial +ve	$Y_x = 3.94 + 3.59x$
P. fluorescence	-0.381	5%	Partial -ve	$Y_x = 62.88 - 8.99x$

Table 4. Estimation of N_2-fixing potential (nitrogenase activity) of isolated diazotroph strains by acetylene reduction assay.

Sl. No	Strain code	Strain identification	*Nitrogenase activity (nM C_2H_2 hr^{-1} mg^{-1} protein)
1.	SDSA-I12/2 (4)	*A. chroococcum*	413.15
2.	SDSA-I14/1 (6)	*A. amazonense*	393.13
3.	SDSA-I30/2 (5)	*B. indica*	364.38
4.	SDSA-I10/1 (3)	*B. caribensis*	402.30
5.	SDSA-I28/1 (2)	*G. liquefaciens*	381.62
6.	SDSA-I19/1(1)	*A. johnsonii*	310.50
7.	SDSA-I28/2 (7)	*D. gummosa*	172.34
8.	SDSA-I22/1 (8)	*B. polymyxa*	181.58
9.	SDSA-I16/1 (9)	*P. fluorescence*	114.80
LSD at 5% significance level			7.74

* Nitrogenase activity is the average of three replicates.

work confirmed the findings of Bhattacharjee *et al.*[16], who have isolated diazotrophs like *Azotobacter* and *Azospirillum* from the paddy fields of South Assam. Isolation of diazotrophic bacteria such as *A. chroococcum, Azospirillum brasilense, A. lipoferum, D. gummosa,* and *B. indica* from the rhizosphere soils of *Cynodon dactylon* (L.) Pers. and *Dichanthium annulatum* (Forsk.) Stapf. by Narolia *et al.,*[17] revealed the occurrence of these strains in the rhizosphere soil of graminaceous plant like rice. Xie *et al.,*[18] isolated *Azospirillum oryzae* sp. *nov.* , a nitrogen fixing bacterium from the roots of rice plant and the present report on occurrence of *A. amazonense* in the vicinity of rice rhizosphere is in conformity with the findings of Xie *et al.,*[19]. Thakuria *et al.,*[20] isolated different *Azospirillum* species such as *A. brasilense, A. amazonense,* and *A. irakense* from the rhizosphere of rice grown in acidic soils of Assam and the occurrence of *A. amazonense* strain in the acidic rice fields of South Assam supported their findings. Occurrence of *A. chroococcum, B. indica* and *D. gummosa* in the rhizosphere of rice grown in acidic soils was reported by many workers[4, 19, 21, 22] over the years is in conformity with the findings of present investigation. The occurrence of N_2-fixing *B. caribensis, G. liquefaciens* and *A. johnsonii* strain in the root region of rice at maturity stage grown in acidic rainfed lowlands of South Assam, India is reported for the first time in the present investigation. Enumeration, isolation and identification of diazotrophs from rhizosphere soil of Korean rice varieties revealed three groups of N_2-fixing bacteria belonging to the genera *Azospirillum, Burkholderia* and *Gluconacetobacter*[23]. Agronomically important paddy rice varieties in Korea harbour different diazotrophic isolates such as *Azospirillum* spp., *Herbaspirillum* spp., *Burkholderia* spp. and *Gluconacetobacter* spp.[23]. Different *Acinetobacter* spp. such as *A. calcoaceticus, A. baumannii, A. lwoffii, A. baylyi* etc. was detected in the rhizosphere of wheat. Prevalence of *Acinetobacter* species in the rhizosphere of wheat was also investigated by a cultivation-dependent approach. *A. calcoaceticus, A. baumannii, A. lwoffii* and *Acinetobacter* sp. were isolated on selective media from the same samples. *In vitro* characterization of *Acinetobacter* isolates revealed that majority of these bacteria exhibited plant growth-promoting traits such as nitrogen fixation, siderophore production and mineral solubilization. These *Acinetobacter* strains may play a favourable role in plant growth promotion while residing in the rhizosphere of wheat[24].

The present work revealed that the number of cultivable N_2-fixing bacteria in the root region of rice at maturity stage in most of the sampling sites of South Assam was over 10^6 CFU g^{-1} dry soil confirming the number found

by Watanabe *et al.*,[25] in paddy soil at the International Rice Research Institute. This may be due to the fact that the number of N_2 fixers is strongly governed by soil organic matter content[19] and rice agro-ecosystem soils of South Assam is rich in organic matter. The population of *Azotobacter* (73 × 10^6), *Azospirillum* (66 × 10^6), *Derxia* (39 × 10^6) and *Beijerinckia* (12 × 10^6) in the rhizosphere of *Cynodon dactylon* was 10^6 CFU g^{-1}dry soil[17]. The population count of *A. chroococcum* was highest followed by *A. amazonense* in the rice field soils of South Assam. The population of *B. indica, B. caribensis, G. liquefaciens* and *A. johnsonii* was of medium range and the CFU count of *D. gummosa, B. polymyxa* and *P. fluorescence* was lowest.

The heavy annual rainfall may cause flooding of rice fields rendering anaerobic condition to native soil diazotrophs and thus reducing the atmospheric N_2 fixation by diazotrophs in the rice field soil. The heavy annual rainfall also lowers the soil pH initiating acid stress to the native diazotrophs of rice fields and the acidity of soil alters the population of diazotrophs. The population of *A. chroococcum, A. amazonense, B. caribensis, G. liquefaciens* and *B. polymyxa* decreases with increase in acidity of rice field soil and that of *B. indica, A. johnsonii, D. gummosa* and *P. fluorescence* increases in acidic rice field soil. The rhizosphere population of *A. chroococcum, A. amazonense, B. caribensis, G. liquefaciens* and *B. polymyxa* showed positive correlation with soil pH accounting higher number in autumn cropping season in the cultivated rice fields of South Assam. The CFU count of *B. indica, A. johnsonii, D. gummosa* and *P. fluorescence* is negatively correlated with soil pH confirming the higher population count of these strains in summer season in the rice fields. All the isolated strains are more or less acid tolerant and therefore they are prevalent in the acidic rice agro-ecosystems of South Assam. In the present investigation it is evident that at increased soil pH in autumn (sali) season the strains of *A. chroococcum, A. amazonense, B. caribensis* and *G. liquefaciens* occurs in higher number whereas the strains of *B. indica, A. johnsonii,* and *D. gummosa* shows maximum population at low soil pH in summer (ahu) season in the rice fields. Among the isolated diazotrophs, *B. indica, A. johnsonii,* and *D. gummosa* were more acid tolerant and may be effective in supplying N_2-nutrition to acidic rice field soils. Higher population of diazotrophs like *A. chroococcum, A. amazonense, B. caribensis* and *G. liquefaciens* in autumn (sali) season may be attributed to increased pH of rice field soil. *B. indica, A. johnsonii,* and *D. gummosa* being acid tolerant strains showed higher population in summer (ahu) season in the rice field soil.

Recently studies are being carried out in different parts of the world about the role of diazotrophic inoculation in crop growth and yield to develop alternative sources of chemical fertilizers. Nitrogenase activity of maize rhizospheric bacteria was detected in 19 isolates ranging from 21.8-3624 nM C_2H_2 hr^{-1} mg^{-1} protein[26]. In the present study, the nitrogenase activity range (114.80-413.15 nM C_2H_4 / hr / mg protein) of isolated diazotroph strains falls within that of rhizospheric bacteria as detected by Naureen *et al.*,[26].

Coinoculation of the strains of *Pseudomonas, Bacillus* and *Azospirillum* on wheat plants resulted high shoot dry weight, total N yield, shoot phosphorus content, nitrogenase activity etc.[27]. *Azospirillum,* PSB and FP isolates increased yield by 27.8-100%, 6.13-21.6% and 28.3-54.7% over the yield of uninoculated control rice plots[28]. Reports showed that seed inoculation of *Azotobacter* sp. on rice have improved growth, nitrogen content, chlorophyll content, grain weight and yield of crops in absence of chemical nitrogen and phosphate fertilizers at its maximum level. The isolated strains of diazotroph are indigenous and best suited to the ecophysiological condition of rice agro-ecosystems of South Assam. Overall, it was evident that the acidic rainfed rice fields of South Assam (Barak Valley) harbour good number of *A. chroococcum, A. amazonense, B. indica, B. caribensis, G. liquefaciens,* and *A. johnsonii* as the major diazotroph strain which have showed higher N_2 fixation rate that and might be used as efficient biofertilizers for growing rice, the major staple food of the people in tropical rainfed acidic lowlands.

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