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Diversity of Soil Diazotrophs in Acid Stress Rice Agroecosystems of Barak Valley of Assam, India

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Six species viz., *Azotobacter chroococcum, Azospirillum amazonense, Beijerinckia indica, Derxia gummosa, Bacillus polymyxa* and *Pseudomonas fluorescence* of diazotrophs were isolated from the acid stress rice agro-ecosystem soils of South Assam (Barak Valley). *Azotobacter chroococcum* and *Azospirillum amazonense* have showed a wide range of distribution in the rice fields with higher number of viable cells. The viable cell count of *Azotobacter chroococcum, Azospirillum amazonense* and *Bacillus polymyxa* strains was more in autumn or sali cropping season (August-December) than in summer or ahu cropping season (May-August). *Beijerinckia indica, Derxia gummosa* and *Pseudomonas fluorescence* strains have shown higher number in summer (ahu) season. The population of diazotrophs fluctuates with the pH of rice agro-ecosystem soil. The population of *Azotobacter chroococcum*, *Azospirillum amazonense* and *Bacillus polymyxa* was high at higher soil pH whereas the population of *Beijerinckia indica, Derxia gummosa* and *Pseudomonas fluorescence* was more at lower soil pH. *Beijerinckia indica* and *Derxia gummosa* strains were more acid tolerant than the other isolated strains. The N_2-fixing potential of *Azotobacter chroococcum* was highest among the isolated diazotrophs. Overall, *Azotobacter chroococcum, Azospirillum amazonense* and *Beijerinckia indica* strains were found to predominate in the rice agro-ecosystem soils of South Assam (Barak Valley).

Key words: Acid stress, Diazotrophs, Diversity, Rice agro-ecosystem, South Assam.

Barak Valley is located in the Southern part of Assam between latitude 24° to 25° North and longitude 92° to 93° East. The valley includes three districts namely Cachar, Karimganj and Hailakandi and is bounded by N. C. hills and Jaintia hills on North, Mizoram in the South, Manipur on East and Tripura and Bangladesh on West. The total geographical area of the zone is 6941.2 sq. km., 8.84% of the total area of the state, Assam. Rice is the major staple food of the people residing in Southern parts of Assam. Rice is cultivated throughout the Barak Valley of South Assam covering maximum portion (about 80%) of cultivable land. The rice is grown once as ahu (summer) crop during May to August and once as sali (autumn) crop during August to December in a calendar year. The average yield of rice (1350 kg/ha) is not as praiseworthy in comparison to the national average, 2691 kg/ha[1]. Rice is not only the staple food of the people; it is also a source of employment and income for the rural population of Barak Valley. To increase the yield of rice farmers are using excessive amount of chemical nitrogenous fertilizers in the field which may result in pollution of soil and water[2]. Excess nitrogen in the global ecosystem in its various forms augments greenhouse effect, diminishes ozone level, promotes smog, contaminates drinking water, acidifies rain, eutrophies river and stresses ecosystems[3]. The use of costly chemical N fertilizers poses a threat to the economy of poverty

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ridden farmers, human health and environment[4]. These problems have renewed public interest in exploring alternate or supplementary nonpolluting source of nitrogen for agricultural production of rice[5]. The N_2 input for a growing rice crop can largely be met by promoting the activities of physiologically diverse microorganisms in the aerobic, anaerobic and interface zones in the ecologically important flooded soils[6].

A range of diazotrophic plant growth promoting rhizobacteria participate in interactions with C_3 and C_4 crop plants (e.g., rice, wheat, maize, sugarcane & cotton) significantly increasing their vegetative growth and grain yield[7]. The tropical lowland rice yield varies from 2-3.5 t/ha using naturally available N derived from biological N_2 fixation by free-living and plant associated diazotrophs and mineralization of soil nitrogen[5]. Diverse free living and associative nitrogen fixing microorganisms (aerobes, facultative anaerobes, heterotrophs, phototrophs) grow in wetland rice fields and contribute to soil N. 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[6]. In addition to N contribution these bacteria also improve the nutrient transformation and contribute to plant growth promoting effects. Therefore, an attempt has been made to study the diversity of soil diazotrophs in acid stress rice agroecosystems of Southern parts of Assam (Barak Valley).

MATERIAL AND METHODS

To study the diazotrophic profile of rice agro-ecosystem soil samples were collected randomly from 18 locations of Cachar district, 14 locations of Karimganj district and 10 locations of Hailakandi district from the rice growing fields during July'2006 to May'2008 representing four cropping seasons; sali(autumn)'2006, ahu(summer)'2007, sali(autumn)'2007 and ahu(summer)'2008. 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 both rhizospheric and nonrhizospheric zones of growing rice crop. The soil samples were placed in sterilized plastic bags, transferred to the laboratory within one day and stored at 4°C prior to further treatment. The samples taken from the same line were combined and each pooled soil sample, henceforward consisting of 20 cores, was homogenized by hand. The largest roots and stones were removed and the soil was mixed carefully. Sterile gloves were used in the soil sampling, working tools were sterilized with ethanol and flamed, and further procedures were also performed as aseptically as possible. Each pooled sample contained about 500 g soils in sterilized plastic bag was stored at 4°C. Isolation of diazotrophs was done within 72 hour of sample collection. Qualitative and quantitative estimation of diazotrophs from all three pooled soil samples of each location (n=3) were carried out by the dilution plate method of Waksman[8, 9]. 10g of moist soil sample was added with 100 ml sterile distilled water in a conical flask and shaken on horizontal shaker to form a homogenous soil suspension. Successive dilutions were made up to 1: 10, 00,000 for the quantitative estimation of viable diazotrophs. 1ml from 1: 10, 00,000 diluted suspensions was transferred aseptically into three sterilized petridishes containing 20ml of melted agar medium. The petridishes were rotated clockwise and anticlockwise to get a homogenous distribution of the inoculums into the medium. The plates were incubated at 27°C ± 2°C. The Burk's Agar medium and Azospirillum Agar medium were used for isolation of diazotrophs. The total number of colony forming units of diazotrophs per gram of soil was counted[10]. The pH of the pooled soil samples was measured following standard method of soil analysis[11]. For qualitative analysis of diazotrophs every type colony was picked up and transferred separately into slant tubes of respective media. Pure culture for each diazotrophs was obtained by streaking agar plates of same medium with inoculums from slant tubes. Identification of diazotrophic bacteria was done by (a) micromorphology (b) cultural, biochemical and physiological characters following Bergey's manual of determinative bacteriology[12]. Micromorphology involved study of cell shape, cell size, cyst formation, flagellation etc. under

olympus trinocular CH-20i microscope. Cultural characteristics involved shape of colony, size of colony, pigmentation, nature of growth and deposition of extracellular substances. Biochemical and physiological studies involved gram reaction, catalase reaction and presence of extracellular gum or intracellular polysaccharide crystals. Nitrogenase activity of isolated diazotrophs was assessed by acetylene reduction test[13]. The technique involved incubation of nitrogenase containing system such as diazotrophs in a known atmosphere of C_2H_2 (10% v/v) in a gas phase and after an optimum time of incubation, C_2H_4 produced is measured by a gas chromatograph using flame ionization detector (FID). Samples were collected in triplicate to conduct the sound statistical analysis. 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[14]. Summary statistics were used to obtain the mean and standard error[15]. The least significant difference and correlation analysis were carried out following the method of Misra and Misra[16].

RESULTS

Altogether, six species of diazotrophs were isolated from the acid stress rice agro-ecosystem soils of South Assam viz., *Azotobacter chroococcum, Azospirillum amazonense, Beijerinckia indica*, *Derxia gummosa, Bacillus polymyxa* and *Pseudomonas fluorescence*. The data in table 1 reveals the population of diazotrophs in rice agro-ecosystem soils of the three districts of Southern Assam in different cropping seasons. In Cachar district, highest population of *Azotobacter chroococcum* ($79 \pm 1.88 \times 10^6$), *Azospirillum amazonense* ($67 \pm 6.67 \times 10^6$), *Bacillus polymyxa* ($36 \pm 2.99 \times 10^6$) and *Pseudomonas fluorescence* ($30 \pm 2.42 \times 10^6$) was found in sali (autumn) season'2007. *Beijerinckia indica* ($52 \pm 5.27 \times 10^6$) and *Derxia gummosa* ($50 \pm 4.14 \times 10^6$) shows maximum population in ahu (summer) season'2008 in the rice fields of Cachar district. The population of *A. chroococcum* ($76 \pm 5.22 \times 10^6$), *A. amazonense* ($70 \pm 2.34 \times 10^6$) and *B. polymyxa* ($32 \pm 3.16 \times 10^6$) was highest in sali (autumn) season'2007 in the rice fields of Karimganj district. The population of *Beijerinckia indica* (46 ± 2.97 X 10^6) and *Derxia gummosa* ($42 \pm 1.87 \times 10^6$) was higher in ahu (summer) season'2008 in the rice agro-ecosystem soils of Karimganj district. The population of *P. fluorescence* ($42 \pm 0.00 \times 10^6$) was higher in ahu (summer) season'2007 in the rice fields of Karimganj district. In Hailakandi district *Azotobacter chroococcum* ($80 \pm 3.90 \times 10^6$) shows maximum number in sali (autumn) season'2006 whereas *Azospirillum amazonense* ($71 \pm 5.61 \times 10^6$) and *Pseudomonas fluorescence* ($22 \pm 0.86 \times 10^6$) has highest population in sali (autumn) season'2007. *Bacillus polymyxa* ($28 \pm 2.81 \times 10^6$) and *Derxia gummosa* ($41 \pm 1.32 \times 10^6$) shows highest number in ahu (summer) season'2007 and *Beijerinckia indica* ($58 \pm 3.23 \times 10^6$) in ahu (summer) seson'2008 in the rice fields of Hailakandi district. On an average the population of diazotrophs was higher in sali (autumn) season than in ahu (summer) season. The population of diazotrophs differed significantly not only between different cropping seasons but between themselves also (Table 2). The seasonal variation of diazotrophic population is highest in the rice fields of Karimganj district. The maximum difference in the population of diazotrophs among themselves was found in the rice fields of Hailakandi district. *Azotobacter chroococcum* population was highest followed by *Azospirillum amazonense* and *Beijerinckia indica*. *Derxia gummosa* was also found in some locations in less number. *Bacillus polymyxa* and *P. fluorescence* population was very less with irregular occurrence.

The climatic condition of Barak Valley favours rice cultivation in tropical lowlands. The Valley 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 temperature and relative humidity are also higher in the summer months between May to August. Among the three districts, maximum annual rainfall and relative humidity occurs in the Karimganj district whereas maximum annual temperature is found in Hailakandi district. All the three districts have a tropical warm humid climate with heavy annual rainfall.

The pH of pooled soil samples of three districts ranges from 4.97 to 5.72 in different cropping seasons. The soil pH decreases with increase in rainfall in the summer (ahu) season effecting the population of diazotrophs in the flooded rice fields of Barak Valley. The acidity of soil decreases in the middle part of autumn (sali) season and consequently the population of diazotrophs increases 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 diazotrophs was correlated with the pH values of pooled soil samples. Table 3 shows that *Azotobacter chroococcum* population is positively correlated with the soil pH which supports its higher population in autumn (sali) season as a result of increased soil pH. *Azospirillum amazonense* and *Bacillus polymyxa* also shows positive correlation with soil pH confirming occurrence of higher number of viable cells per gram of soil in autumn (sali) season in the rice agro-ecosystem of Barak Valley. The population of *Beijerinckia indica, Derxia gummosa* and *Pseudomonas fluorescence* shows negative correlation with the soil pH indicating occurrence of lower number of viable cells per gram of soil at increased soil pH in autumn (sali) season.

The present experiment revealed that soil pH effects the population of diazotrophs in the rice agro-ecosystem of Barak Valley. *Azotobacter chroococcum, Azospirillum amazonense* and *Bacillus polymyxa* shows increase in viable cell count with concomitant increase in soil pH but *Beijerinckia indica, Derxia gummosa* and *P. fluorescence* population decreases with increasing soil pH. Since the soil pH is high in autumn (sali) season, diazotrophs like *A. chroococcum, A. amazonense* and *Bacillus polymyxa* shows higher number of viable cells in the rice fields. In summer (ahu) season the soil pH decreases due to heavy rainfall and acid tolerant diazotrophs like *B. indica, D. gummosa* and *P. fluorescence* shows thick population in the rice fields.

The isolated diazotrophs were identified by cultural, micromorphological, biochemical and physiological characteristics following Bergey's manual of determinative bacteriology. *Azotobacter chroococcum* strains were 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,

Table 1. Quantitative estimation of soil diazotrophs in the rice agro-ecosystems of three districts of South Assam in different cropping seasons

District	Soil diazotroph	*Number of cells / g of dry soil (× 10^6)			
		Sali season'06	Ahu season'07	Sali season'07	Ahu season'08
Cachar	*Azotobacter chroocdoccum*	63 ± 2.40	38 ± 2.09	79 ± 1.88	39 ± 2.46
	Azospirillum amazonense	56 ± 3.04	31 ± 2.40	67 ± 6.67	35 ± 2.02
	Beijerinckia indica	15 ± 1.32	41 ± 3.65	8 ± 1.20	52 ± 5.27
	Derxia gummosa	12 ± 0.28	37± 0.78	21 ± 0.95	50 ± 4.14
	Bacillus polymyxa	26 ± 2.78	30 ± 3.90	36 ± 2.99	28 ± 1.81
	Pseudomonas fluorescence	0	18 ± 0.00	30± 2.42	29 ± 2.20
Karimganj	*Azotobacter chroococcum*	59 ± 2.26	38 ± 2.43	76 ± 5.22	37 ± 2.20
	Azospirillum amazonense	67 ± 2.96	21 ± 2.84	70 ± 2.34	26 ± 2.02
	Beijerinckia indica	0	28 ± 1.47	18± 1.25	46 ± 2.97
	Derxia gummosa	18 ± 0.80	36 ± 3.00	42 ± 1.87	0
	Bacillus polymyxa	23 ± 3.29	27 ± 3.21	32 ± 3.16	12 ± 0.00
	Pseudomonas fluorescence	22 ± 1.12	42 ± 0.00	0	11 ± 0.87
Hailakandi	*Azotobacter chroococcum*	80 ± 3.90	48 ± 2.00	73 ± 2.81	43 ± 4.80
	Azospirillum amazonense	68 ± 3.10	35 ± 3.04	71 ± 5.61	38 ± 1.98
	Beijerinckia indica	21 ± 2.12	44 ± 4.87	15 ± 0.00	58 ± 3.23
	Derxia gummosa	26 ± 1.71	41 ± 1.32	19 ± 0.90	32 ± 1.90
	Bacillus polymyxa	23 ± 0.85	28 ± 2.81	26 ± 2.35	0
	Pseudomonas fluorescence	18 ± 1.42	0	12± 1.12	22 ± 0.86

± SeM, * No. of cells/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.

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. The bacterial strains obtained on *Azospirillum* medium from rice agro ecosystem soil were identified as

Table 2. Analysis of variance of the data on quantitative estimation of soil diazotrophs in the rice agroecosystems of the three districts of South Assam

District	Source of variation	Degree of freedom	Calculated F	Tabulated F	Probability level	Significance level
Cachar	Between seasons	$n_1 = 3$ $n_2 = 15$	7.84	3.3	0.05	**
	Between diazotrophs	$n_1 = 5$ $n_2 = 15$	5.53	2.9	0.05	**
Karimganj	Between seasons	$n_1 = 3$ $n_2 = 15$	13.89	3.3	0.05	***
	Between diazotrophs	$n_1 = 5$ $n_2 = 15$	6.59	2.9	0.05	**
Hailakandi	Between seasons	$n_1 = 3$ $n_2 = 15$	7.91	3.3	0.05	**
	Between diazotrophs	$n_1 = 5$ $n_2 = 15$	12.57	2.9	0.05	***

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

Table 3. Correlation analysis of the population of diazotrophs and soil pH in the rice agro-ecosystems of three districts of South Assam

Diazotrophs	Calculated value of r at 11 degree of freedom	Significance level	Type of correlation	Regression equation
Azotobacter chroococcum	0.970	0.1%	Perfect +ve	Yx = -101.21 + 27.79x
Azospirillum amazonense	0.966	0.1%	Perfect +ve	Yx = -124.38 + 30.59x
Beijerinckia indica	-0.759	0.1%	Perfect -ve	Yx = 182.67 – 27.83x
Derxia gummosa	-0.092	5%	Partial -ve	Yx = 31.37 – 2.57x
Bacilluspolymyxa	0.229	5%	Partial +ve	Yx = 3.94 + 3.59x
Pseudomonas fluorescence	-0.381	5%	Partial -ve	Yx = 62.88 – 8.99x

A. amazonense 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. *Beijerinckia indica* strains were confirmed on the basis of their 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. *Derxia gummosa* strains 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, intracellular polysaccharide crystals and extracellular gum. The genus *P. fluorescence* shows 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 diazotrophs were tested for the ability to fix

atmospheric N_2 by acetylene reduction technique. In this technique the activity of nitrogenase enzyme of diazotrophs was assessed for its capacity to reduce acetylene to ethylene. The more the activity of nitrogenase enzyme the greater is the ability of atmospheric N_2-fixation. The maximum nitrogenase activity was shown by *Azotobacter chroococcum* culture (413.15 nM C_2H_4/ hr / mg protein). *Azospirillum amazonense* and *Beijerinckia indica* cultures also have good N_2-fixation ability. *Derxia gummosa, Bacillus polymyxa* and *Pseudomonas fluorescence* culture has less activity of nitrogenase enzyme indicating poor nitrogen fixing ability (Table 4).

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

Sample No.	Isolated diazotroph	*Nitrogenase activity (nM C_2H_4 hr^{-1} mg^{-1} protein)
1.	*Azospirillum amazonense*	393.13
2.	*Bacillus polymyxa*	181.50
3.	*Azotobacter chroococcum*	413.15
4.	*Derxia gummosa*	272.30
5.	*Beijerinckia indica*	300.30
6.	*Pseudomonas fluorescence*	114.80
	LSD at 5% significance level	4.94

* Nitrogenase activity is the average of three replicates.

DISCUSSION

In the present investigation six species of diazotrophs viz., *Azotobacter chroococcum*, *Azospirillum amazonense, Beijerinckia indica*, *Derxia gummosa, Bacillus polymyxa and Pseudomonas fluorescence* were isolated from the paddy fields of South Assam. Bhattacharjee *et al.*[17] (2001) isolated diazotrophs like *Azotobacter* and *Azospirillum* from the paddy fields of Cachar district. This finding supported the occurrence of diazotrophs in the rice fields of South Assam (Barak Valley). Diazotrophic bacteria such as *Azotobacter chroococcum, Azospirillum brasilense, Azospirillum lipoferum, Derxia gummosa, Beijerinckia indica* were isolated from the rhizosphere soils of *Cynodon dactylon* (L.) Pers. and *Dichanthium annulatum* (Forsk.) Stapf. by Narolia *et al.*[18] also supported the occurrence of *Azotobacter chroococcum, Azospirillum amazonense, Beijerinckia indica* and *Derxia gummosa* strains in the rhizosphere soil of graminaceous plant like rice. *Azospirillum oryzae* sp. nov. , a nitrogen fixing bacterium isolated from the roots of the rice plant *Oryzae sativa* supported the occurrence of *Azospirillum* species in the vicinity of rice rhizosphere in present investigation[19]. Thakuria *et al.*[1] (2004) isolated different *Azospirillum* species such as *Azospirillum brasilense*, *Azospirillum amazonense*, and *Azospirillum irakense* from the rhizosphere of rice grown in acidic soils of Assam supports the occurrence of *Azospirillum amazonens*e strains in the acidic rice fields of South Assam. Occurrence of *Azotobacter chroococcum*, *Beijerinckia indica* and *Derxia gummosa* in the rhizosphere of rice grown in acidic soils was reported by many workers[6, 7] over the years confirm the findings of present investigation. The population of *Azotobacter chroococcum* (73×10^4), *Azospirillum amazonense* (66×10^4), *Derxia gummosa* (39×10^4) and *Beijerinckia indica* (12×10^4) in the rhizosphere soil of *Cynodon dactylon* was higher than that in the rhizosphere soil of *Dichanthium annulatum*[18]. In the present investigation the population of *Azotobacter chroococcum* was highest followed by *Azospirillum amazonense* in the rice field soils of Southern Assam. The population of *Beijerinckia indica* and *Derxia gummosa* was of medium range. *Bacillus polymyxa* and *Pseudomonas fluorescence* showed lower population in the rice fields. These findings were supported by the experiment of Narolia *et al.*[18]. 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 *Azotobacter chroococcum, Azospirillum amazonense* and *Bacillus polymyxa* decreases with increase in acidity of rice field soil and the population of *Beijerinckia indica*, *Derxia gummosa* and *Pseudomonas fluorescence* increases in acidic rice field soil. Soil diazotrophs have been known to be greatly influenced by soil type and physico-chemical properties of soil [8]. Kimura *et al.*[21] studied extensively the rhizosphere

soil of paddy in Japan with stress on the effect of anaerobiosis on microbes. The soil pH in the rice fields of Southern Assam was more or less similar with the findings of Takeuchi and Hayano[22] in Japan. They characterized a rice field soil under monoculture of rice. According to them the soil pH was 6.6 in the rice agro-ecosystem soils of Japan. In the present investigation it is evident that at increased soil pH in autumn (sali) seasons the strains of Azotobacter *chroococcum, Azospirillum amazonense* and *Bacillus polymyxa* occurs in higher number whereas the strains of *Beijerinckia indica, Derxia gummosa* and *Pseudomonas fluorescence* shows maximum population at low soil pH in summer (ahu) season in the rice fields. Among the isolated diazotrophs *Beijerinckia indica* and *Derxia gummosa* were more acid tolerant and can be effective in supplying N_2-nutrition to acidic rice field soils. Higher population of diazotrophs like *Azotobacter chroococcum, Azospirillum amazonense* and *Bacillus polymyxa* in autumn or sali season may be attributed to increased pH of rice field soil. *Beijerinckia indica*, *Derxia gummosa* and *Pseudomonas fluorescence* being acid tolerant strains showed higher population in summer or ahu season in the rice field soil. All the isolated strains are more or less acid tolerant and therefore they are prevalent in the acid stress rice agro-ecosystems of South Assam.

Nitrogenase activity of maize rhizospheric bacteria was detected in 19 isolates ranging from 21.8-3624 nM C_2H_4 produced / hr / mg protein[20]. In the present investigation the nitrogenase activity range (114.80-413.15 nM C_2H_4 / hr / mg protein) of isolated diazotrophs falls within that of rhizospheric bacteria as detected by Naureen *et al.*[20].

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. 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.[23]. *Azospirillum*, P SB 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 [24]. 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 diazotrophs are native to the rice field soils and best suited to the ecophysiological condition of rice agro-ecosystems of Southern Assam. Identification and screening of these isolated strains of diazotrophs for their ability to improve the growth and yield of rice will be useful in developing native strains of biofertilisers for the most cultivated crop rice in Southern parts of Assam.

CONCLUSION

The North Eastern part of India and particularly Southern parts of Assam is not self sufficient in food grain production and the people of this area are mostly depended on the supply of food grains from other states. The main diet of people in this area is rice and its production is to be increased with the increasing annual demand. Isolation of native strains of soil diazotrophs will explore the N_2-fixing bacterial diversity in the soil which can be used as biofertilisers in rice cultivation. This will help in reducing the use of costly and hazardous chemical fertilizers in the cultivation of rice. *Azotobacter chroococcum, Azospirillum amazonense, Beijerinckia indica, Derxia gummosa, Bacillus polymyxa* and *Pseudomonas fluorescence* were the six species of diazotrophs isolated from the rice field soils. Among these species *Azotobacter chroococcum* (57-100%) and *Azospirillum amazonense* (38-90%) showed maximum frequency in the rice agro-ecosystem soil. The relative abundance of *Azotobacter chroococcum* (45%) is highest followed by *Azospirillum amazonense* (25%) and *Beijerinckia indica* (10%). Occurrence of *Derxia gummosa, Bacillus polymyxa and Pseudomonas fluorescence* was thin with low frequency and relative abundance. Overall, it is evident that the acidic rice field soils of Southern parts of Assam harbour good number of *Azotobacter chroococcum*, *Azospirillum amazonense* and *Beijerinckia indica* as the major diazotroph with higher frequency and relative abundance showing potential for N_2 fixation that can be developed as efficient strains of biofertilisers for growing rice,

the major staple food of the people in Southern parts of Assam (Barak Valley).

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