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Biodiversity of soil diazotrophs in acid stress rice agroecosystem of southern Assam

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Considering the role of soil diazotrophs in N_2 nutrition of cultivated crops an experiment was carried out to study the biodiversity of soil diazotrophs in acid stress rice agro ecosystem of Southern Assam. The rice field soils were collected randomly from 18 different localities of three districts of Southern Assam and its adjoining areas during *Sali* season 2007 in the month of October- November in pre harvest condition. The soil dilution plate technique was adopted for isolation of soil diazotrophs. Altogether four genera of soil diazotrophs viz., *Azotobacter chroococcum, Azospirillum amazonense, Beijerinckia indica and Derxia gummosa* were isolated. The most abundant diazotroph was *Azotobacter chroococcum* with a maximum population density.

Key words: Soil diazotrophs, acid stress, rice agro ecosystem, biofertilizers

INTRODUCTION

Nitrogen is an essential plant nutrient and its deficiency in soil results in reduced agricultural yields throughout the world. To supply the nitrogen requirement of a crop excessive amount of chemical nitrogenous fertilizers are being used globally. But, keeping in view the forthcoming energy crisis and adverse effects of chemical nitrogenous fertilizers on our global ecosystem and human health, there started an effort of reducing the use of chemical nitrogenous fertilizers in agriculture. The biological sources of nutrients are gaining importance over chemical and organic sources from the standpoint of environmental safety and quality, and sustainable agriculture (Rao *et al,* 1998). This trend has intensified efforts all over the world to study the biodiversity of soil diazotrophs which can harvest atmospheric nitrogen and solubilise phosphorus available in the soil. In natural ecosystems biological nitrogen fixation by free-living, associated and symbiotic diazotrophs is the most important source of nitrogen (Cleveland *et al.,* 1999; Paul and Clark, 1996). The degree of soil acidity or alkalinity influences the population of soil diazotrophs. The acid soil of humid regions harbours less number of soil diazotrophs whereas alkaline soil favours a higher number of soil diazotrophs in general (Waksman and Starkey, 2006). Considering the potential of soil diazotrophs in nitrogen nutrition of cultivated crops an experiment has been carried out to explore the biodiversity of diazotrophs in acid stress rice field soil of Southern parts of Assam and its adjoining areas.

MATERIALS AND METHODS

The rice field soil samples were collected randomly from different locations of Southern Assam and its adjoining areas during *Sali* season, 2007 in the month of October- November in pre harvest condition as per the procedure outlined by Dobereiner *et al.* (1976) to study the biodiversity of soil diazotroph of rice agro ecosystem. The collected soil samples were kept in sterilized polybags after proper tagging. The soil samples were collected from 18 locations throughout three districts viz., Cachar, Karimganj and Hailakandi of Southern Assam and its adjoining areas. The soil of Southern parts of Assam varied from alluvial to laterite type. Texture was generally clay loam to clay. The pH of rice field soils of Southern Assam ranged from 4.5 to 6.0 pointing to the acidic nature of the soil and this fact was attributed to heavy precipitation in the area (Anonymous, 2007). The soil diazotrophs were isolated on N_2-free Burk's enrichment agar medium

by dilution plate method. The isolates of soil diazotrophs were purified by streak plate and pour plate methods in succession. The isolates of soil diazotrophs were identified on the basis of cultural, morphological and biochemical characteristics. The colonies of different diazotrophs were counted with a colony counter on N_2-free Burk's enrichment medium and the data were recorded.

RESULTS AND DISCUSSION

The experimental results were shown in the Tables 1 and 2 and it revealed that soils of this region composed of good number of soil diazotrophs which were isolated from the six soil samples for each district of Southern Assam. The purified isolates were examined for the cultural, morphological and biochemical characteristics and four genera of soil diazotrophs were identified. Out of 40 isolates, 16 isolates are of the genus *Azotobacter,* 12 isolates are of the genus *Azospirillum,* 7 isolates are of the genus *Beijerinckia* and 5 isolates are of the genus *Derxia.* All these isolates were identified as per the Bergey's manual of systematic bacteriology (Krieg, 1984).

The data in Table 1 show the number of colonies of soil diazotrophs isolated on Burk's medium and the population of diazotrophs per gram of soil. Number of colonies of soil diazotrophs in a particular district was the average of all the six soil samples of the district. Altogether four different *strains* of diazotrophs, viz. *Azotobacter, Azospirillum, Beijerinckia, Derxia,* were isolated from rice agroecosystem of this region. Only three genera of soil diazotrophs, viz., *Azotobacter, Azospirillum* and *Derxia* were reported from the rice fields of Cachar district and among the three genera, Azotobacter had showed highest number of cells per gram of soil (67.39×10^4). In Karimganj district the four genera of soil diazotrophs isolated included, *Azotobacter, Azospirillum, Beijerinckia* and *Derxia* with Azotobacter having a maximum number of cells per gram of soil (52.18×10^4). *Azotobacter, Azospirillum, Beijerinckia* and *Derxia* were the four genera of soil diazotrophs found in the rice fields of Hailakandi district with *Azotobacter* showing the highest cell count per gram of soil (55.50×10^4). Overall, the population density of *Azotobacter* was maximum in the rice field soils of Southern Assam followed by *Azospirillum* which was predominant in flooded low lying rice fields. *Beijerinckia* and *Derxia* strains have shown their thin presence in the rice root soil vicinity. *Derxia* strains have the lowest number of cells per gram of soil in all the districts. The optimum pH for growth and nitrogen fixation by *Azotobacter* was 7-7.5 but in presence of combined nitrogen the pH range for growth was 4.8-8.5. *Azospirillum* could grow between pH 5 to pH 7.5 whereas *Beijerinckia* grew between pH 3 to pH 9. *Derxia* grew between pH 5.5 to pH 9. It was mentioned earlier that the soil pH of rice fields of Southern Assam was below 6.0 which supports the occurrence of acid tolerant strains of diazotrophs like *Azotobacter chroococcum, Azospirillum amazonense, Beijerinckia indica,* and *Derxia gummosa* (Subbarao, 1988; Andre *et al.* 2004).

Table 1: Quantitative analysis of soil diazotrophs in acid stress rice field soil of Southern Assam

Districts	Isolated diazotroph	No. of colonies*	Avg. no. of cells per gram of soil
Cachar	*Azotobacter*	67.39	67.39×10^4
	Azospirillum	47.33	47.33×10^4
	Derxia	22.70	22.70×10^4
Karimganj	*Azotobacter*	52.18	52.18×10^4
	Azospirillum	41.23	41.23×10^4
	Beijerinckia	30.07	30.07×10^4
	Derxia	27.05	27.05×10^4
Hailakandi	*Azotobacter*	55.50	55.50×10^4
	Azospirillum	53.75	53.75×10^4
	Beijerinckia	47.33	47.33×10^4
	Derxia	8.75	8.75×10^4

*No. of colonies is the average of six replicates.

Table 2 : Biological parameters of isolated soil diazotrophs

Soil diazotroph	Cultural characteristics	Nature of growth	Gram reaction	Catalase reaction	Cell shape	Cell size	Flagellation	Polysaccharide/g production	Cyst formation
Azotobacter	Spherical gummy opaque colony	Aerobic	Negative	Positive	Large ovoid or rod shaped cell	2x1.5 µm	Flagella present	Produces extra cellular slime	Thick walled cysts
Azospirillum	Subsurface white pellicle in semisolid medium	Microaerophilic/aerobic	Negative	Variable	Cells rod shaped or vibroid	2.5 x 0.5 µm	Flagella present	Polysaccharide crystals in cytoplasm	Cyst absent
Beijerinckia	Giant colony with folded surface with copious, tenacious & elastic slime.	Aerobic	Negative	Positive	Rod shaped with rounded ends.	3.5 x 1.2 µm	Flagella present	Polysaccharide granules on each pole.	Cyst absent
Derxia	Thin, whitish colonies with gum deposition.	Aerobic	Negative	Negative	Curved rods with rounded ends.	4 x 1.5 µm	Flagella present	Older cells have refractile granules in cytoplasm.	Cyst absent.

The data in Table 2 showed the biological parameters of isolated soil diazotrophs. It revealed the cultural, morphological and biochemical characteristics of the diazotrophs. The 16 isolated strains were identified to be the strains of *Azotobacter chroococcum* on the basis of characteristics like spherical gummy opaque colony, large ovoid or rod shaped cells, cells occurred singly or in chains, motile, presence of thick walled cysts, gram negative, catalase positive and aerobic. The 12 isolated strains were identified to be the strains of *Azospirillum amazonense*, from the observed characteristics like subsurface white pellicle in semisolid medium, cells rod shaped or vibroid, motile, presence of polysaccharide crystals in the cytoplasm, gram negative, catalase variable, microaerophilic or aerobic. The 7 isolates obtained on Burk's medium were identified as species of *Beijerinckia indica* on the grounds that they showed smooth, shining and white giant colony, single rod shaped cell with rounded ends, motile, aerobic, gram negative, catalase positive, presence of polysaccharide granules on each pole of cell. The 5 isolates of the genus *Derxia* isolated from the acid stress rice fields of Southern Assam were identified as strains of *Derxia gummosa* on the basis of characteristics such as thin whitish colonies with gum deposition, rod shaped cell with rounded ends, gram negative, motile, aerobic, catalase negative and cells had retractile granules in the cytoplasm.

All these strains of soil diazotrophs isolated from the acid stress rice field soils of Southern Assam involved free-living and associative diazotrophs which could be developed as efficient strains of bio fertilizers for cultivated crops in Southern parts of Assam. The role of diazotrophs in crop yield was documented by Okon and Kapulnik (1986), Ramasamy *et al.* (1992) and Srinivasan and Prabakaran (1992). In rice, nearly 70 kg of N ha^{-1} crop could be derived through rice root associated biological nitrogen fixation. Since 20-25% of nitrogen needs of rice can be supplemented from associative fixation (Deiveekasundaram, 2006) the isolated diazotrophs could be used in supplementing the nitrogen needs for rice in Southern Assam. Now a day's organism capable of fixing atmospheric nitrogen like *Rhizobium, Azotobacter* and *Azospirillum* were commercially produced as biofertilizers to supply nitrogen and phosphate solubilising organisms were mass multiplied as biofertilizers aiding phosphorus nutrition (Bagyaraj, 2003). Application of biofertilizers also substantially improved soil fertility status by increasing the nitrogen, phosphorus, and organic carbon (Muthukumaravel *et al*, 2006). To supply the N_2 nutrition of cultivated crops like rice in Southern parts of Assam the N_2-fixing potential of native strains of free-living and associative soil diazotrophs could be exploited. Rahman *et al.* (2004), reported that inoculation of efficient and better N_2-fixing *Azotobacter* strains could increase rice productivity and therefore there was a great potential of free-living and associative soil diazotrophs in supplying the N_2 nutrition for cultivated crops like rice. Further, characterization and maintenance of native strains of soil diazotrophs will help in developing better and superior strains of biofertilizer to be used in sustainable nutrient. management programme for cultivated crops like rice in Southern parts of Assam and its adjoining areas.

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