SUMMARY
The North Eastern part of India and particularly, Southern part of Assam (Barak valley) is not self-sufficient in food grain production and the people of this area are mostly dependent on the supply of food grains from other states. Due to geographical location the supply of food grains to this area becomes costly and the price of food materials stands very high. Since the economic condition of majority of the population of this zone is not healthy they are facing a problem of food security. The main diet of people in this area is rice and its production has to be increased in parallel with the increasing annual demand. Since biofertilizers has made rapid strides during the last 15 years or so in India, it is very much essential to derive the benefits of biofertilization in this region of the country. Now a day, biofertilizer research is a thrust area of agriculture both in and outside the country to supply the future demand of food grains in a sustainable way. Therefore, the present work is not only very significant from the perspective of modern agriculture and future food security but also it may upgrade the status of biofertilizer utilization and management in South Assam.

This research work was undertaken to isolate the indigenous strains of N₂-fixing bacteria from tropical rainfed flooded rice agro-ecosystems and to screen the isolated diazotrophs for biofertilization of field grown rice. Isolation of diazotrophs was done from different agricultural blocks of the three districts of South Assam. In this zone, rice is mostly grown twice in a year, once as autumn (ahu) crop during April to August and once as winter (sali) crop during August to December. In most of the seasons autumn crop suffers from flooding and therefore, farmers of the zone gives more emphasis on winter crop. The strains were isolated once each in autumn (ahu) and winter (sali) season from the rhizosphere and nonrhizosphere soils of 30 to 60 days old field grown rice. In Barak Valley, rice is mostly grown in the flooded lowlands and the annual production is not as praiseworthy as it was expected. One of the major causes of low rice production of Northeast India and South Assam, in particular, is the devastating flooding of the rice fields in autumn season. Flood water inundates the growing rice plants and destroys the rice crop. Flooding, thus imbalances the economy of the poverty ridden people of South Assam and farmers in particular.

The annual production of rice in Assam is decreasing in recent years. One of the possible causes of this decline in the productivity of rice is degradation of soil health by excessive use of chemical fertilizers. Uncontrolled and excessive use of chemical fertilizers renders the soil unfavourable for the survival of agriculturally beneficial microorganisms such as bacteria, cyanobacteria, actinomycetes, fungi etc. Therefore, need arises to supplement the use of chemical fertilizers with nonpolluting, eco-friendly and low cost biofertilizers. The rice fields of South Assam
are rich in organic matter but deficient in inorganic nitrogen, the chief plant growth promoting nutrient. Therefore, efforts have been made to isolate the indigenous strains of N₂-fixing bacteria from the rice fields and to use the isolated strains as biofertilizers for growing rice.

The Southern part of Assam is characterized by a tropical warm and humid climate. The annual rainfall is well over 2500 mm which causes leaching of applied fertilizers and lowers the availability of nutrients to plants. High annual rainfall also lowers the soil pH and this renders acid stress to the native strains of diazotrophs. In this situation only those diazotroph strains which have the capacity for survival at lower pH level can persist in the soil and supply nitrogen to the growing rice crop. It was observed that *B. indica*, *D. gummosa*, *A. johnsonii* and *P. fluorescence* strains have better tolerance to low soil pH. *A. chroococcum*, *A. amazonense*, *B. caribensis*, *G. liquefaciens* and *B. polymyxa* strains showed higher population in winter season as soil pH increases slightly in comparison to autumn season. The rice fields of South Assam harbour acid tolerant strains of *B. indica*, *D. gummosa*, *A. johnsonii* and *P. fluorescence* as dominant diazotrophs in autumn (ahu) season whereas in winter (sali) season strains of *A. chroococcum*, *A. amazonense*, *B. caribensis*, *G. liquefaciens* and *B. polymyxa* have shown prevalence. This finding suggested that the strains of *B. indica*, *D. gummosa*, *A. johnsonii* and *P. fluorescence* can be used as microbial fertilizer for growing autumn rice in alluvial flood plains of South Assam. *A. chroococcum*, *A. amazonense*, *B. caribensis*, *G. liquefaciens* and *B. polymyxa* strains can be utilized as microbial inoculants for growing winter rice keeping in view their prevalence in rice field soils.

The rice agro-ecosystem soil of South Assam is alluvial to laterite type. The texture is generally clay loam to clayey. The pH is slightly acidic and EC is of medium range. The soil is rich in organic matter but deficient in inorganic N content due to leaching losses of applied N fertilizers caused by heavy annual rainfall. The rich organic matter content made the soil favourable for growth of a range of microorganisms including diazotrophs. The potassium availability of the soil is of medium range and the phosphorus content is low. The climatic condition of Northeast India favour rice cultivation and if it become possible to map soil health of different districts, it will be easier to choose the suitable microbial fertilizer to the ecophysiological condition of soil of that district. Use of regional specific strains of diazotroph will be helpful to boost the rice productivity of the region in a sustainable way.

Nine species of heterotrophic diazotroph were isolated from the acidic rice field soils of South Assam. The diazotroph species were identified as *A. chroococcum*, *A. amazonense*, *B. indica*, *B. caribensis*, *G. liquefaciens*, *A. johnsonii*, *D. gummosa*, *B. polymyxa* and *P. fluorescence*. The population of *A. chroococcum*, *A. amazonense*, *B. caribensis*, *G. liquefaciens*, and *B. polymyxa* was higher in winter season whereas *B. indica*, *A. johnsonii*, *D. gummosa*, and *P. fluorescence* have shown higher population in autumn season. On an average, the population of *A. chroococcum* was highest.
followed by *A. amazonense* in the rice fields. The population of *B. indica*, *B. caribensis*, *G. liquefaciens*, and *A. johnsonii* was of medium range. *D. gummosa*, *B. polymyxa*, and *P. fluorescence* have shown lower population in rice fields. The heavy annual rainfall causes flooding of rice fields rendering anaerobic condition to native diazotroph strains and thus, reducing atmospheric N₂-fixation. The seasonal variation in the diazotroph population is due to change in soil pH and moisture content. The population of *A. chroococcum*, *A. amazonense*, *B. caribensis*, *G. liquefaciens*, and *B. polymyxa* decreased with increase in soil acidity and that of *B. indica*, *A. johnsonii*, *D. gummosa*, and *P. fluorescence* increased with simultaneous increase in soil acidity. *A. chroococcum*, *A. amazonense*, *B. caribensis*, *G. liquefaciens*, and *B. polymyxa* strains were dominant in winter season at higher soil pH and low moisture content.

On assaying the nitrogenase activity of isolated diazotroph strains, it was observed that *A. chroococcum*, *B. caribensis*, *A. amazonense*, *G. liquefaciens*, *B. indica* and *A. johnsonii* strains have higher activity of nitrogenase enzyme. The more the activity of nitrogenase enzyme the higher is the capacity to fix atmospheric nitrogen. Thus, it was evident that the acidic rice field soils of South Assam harbour good number of *A. chroococcum*, *B. caribensis*, *A. amazonense*, *G. liquefaciens*, *B. indica* and *A. johnsonii* strains as the major diazotroph population with higher frequency and relative abundance which have shown higher atmospheric dinitrogen fixing ability. Therefore, these strains were used as inoculants for growing rice in autumn (ahu) and winter (sali) seasons in absence of chemical fertilizers.

Four nitrogen fixing strains namely, *Azospirillum amazonense*, *Gluconacetobacter liquefaciens*, *Beijerinckia indica* and *Dexia gummosa* of the class α-proteobacteria; one strain of *Burkholderia caribensis* of the class β-proteobacteria; three strains namely, *Azotobacter chroococcum*, *Acinetobacter johnsonii* and *Pseudomonas fluorescence* of the class γ-proteobacteria and one strain of *Bacillus polymyxa* belonging to the class bacilli were isolated and characterized from the rice agro-ecosystems of South Assam. *Burkolderia caribensis*, a nitrogen fixing bacterium was reported for the first time in the rhizosphere and nonrhizosphere region of rice grown in acidic flooded lowland agro-ecosystem of South Assam. *Acinetobacter johnsonii*, a pathogenic bacterium have been found to occur as a free-living heterotrophic nitrogen fixer in the nonrhizosphere region of rice grown in acidic flooded rice fields of South Assam.

The 16S rDNA nucleotide sequences of the six selected strains of diazotroph isolated from the rice agro-ecosystems on the basis of their N₂-fixing potential were deposited in NCBI GenBank. The six strains viz., *B. caribensis* SDSA-110/1, *A. johnsonii* SDSA-119/1, *G. liquefaciens* SDSA-128/1, *A. amazonense* SDSA-114/1, *B. indica* SDSA-130/2, and *A. chroococcum* SDSA-112/1 have been
assigned NCBI GenBank accession numbers GU372342, GU372343, GU372344, GU372345, GU372346, and GU372347 respectively.

It was observed that apart from diazotrophy the above strains could be better exploited as inoculants since they possess other PGP characteristics, in particular, IAA production, P-solubilization and ACC deaminase activity. Diazotroph inoculation of rice seedlings increased plant height, shoot length, root length, number of tillers/plant, plant fresh weight, weight of 100 grains, dry biomass/plant, grain yield, chlorophyll\textsubscript{a} content of leaves, N content of shoot, protein content of grains and root NR activity by 5-41\%, 4-37\%, 2-28\%, 4-43\%, 5-30\%, 7-30\%, 11-49\%, 5-35\%, 9-46\%, 8-47\%, 8-37\%, and 8-27\% respectively over uninoculated control plants in autumn and winter season. Indigenous strains of heterotrophic free-living, associative and/or endophytic diazotrophs namely, \textit{A. chroococcum}, \textit{A. amazonense}, \textit{B. indica}, \textit{B. caribensis}, \textit{G. liquefaciens}, and \textit{A. johnsonii} isolated from the rhizosphere and nonrhizosphere region of low lying acidic rice fields are efficient N\textsubscript{2}-fixers and possess several other PGP traits like IAA production, P- and Zn-solubilization, S-oxidation, ACC deaminase activity, siderophore production etc. Inoculation of these strains improved plant height, shoot length, root length, tiller number, fresh and dry biomass, grain yield, chlorophyll\textsubscript{a} content, N-content of shoot, protein content of grains, NR activity of root and activity of root nitrogen assimilatory enzymes (GS, GOGAT and GDH) significantly over uninoculated control plants of both autumn and winter rice under field condition. Vermicompost can be used as effective carrier material for \textit{A. chroococcum}, \textit{A. amazonense}, \textit{B. caribensis}, and \textit{G. liquefaciens} as it provided suitable micro-environment for better survival of these diazotroph strains and vermicompost based inoculant of these strains enhanced growth, N-uptake and yield of autumn (ahu) rice (cv. IR-36) in acidic flooded lowlands. The charcoal based preparation of \textit{B. indica} and \textit{A. johnsonii} has shown higher survival of inoculant bacteria and charcoal based inoculant of these bacteria resulted significant improvement in growth and yield parameters of rice which suggests that charcoal can be used as efficient carrier of \textit{B. indica} and \textit{A. johnsonii} for inoculation of rice seedlings grown in acidic lowlands.

The major findings of this research work are:-

Application of chemical N fertilizer in growing the major staple food, rice in Southern parts of Assam might cause economic losses of farmers, environmental and health hazards, pollution of water ecosystems, loss of indigenous strains of diazotroph associated with the traditional rice varieties, and limited annual rice yield due to leaching losses of applied chemical fertilizers under heavy rainfall condition in flooded lowland rice ecosystems.

Nine strains of culturable diazotrophs were isolated from the acid stress rice agro-ecosystems viz., \textit{Azotobacter chroococcum} (SDSA-I12/1), \textit{Azospirillum amazonense} (SDSA-I14/1), \textit{Beijerinckia indica} (SDSA-I30/2), \textit{Burkholderia caribensis} (SDSA-I10/1), \textit{Gluconacetobacter liquefaciens} (SDSA-I28/1).
Acinetobacter johnsonii (SDSA-119/1), Derxia gummosa (SDSA-128/2), Bacillus polymyxa (SDSA-122/1) and Pseudomonas fluorescence (SDSA-116/1) which are the indigenous heterotrophic free-living, associative and/or endophytic N_2-fixing bacteria best suited to the ecophysiological condition of the lowland rice ecosystems of South Assam.

Burkholderia caribensis, a nitrogen fixing bacterium was reported for the first time from the rhizosphere and nonrhizosphere region of rice grown in tropical rainfed flooded lowlands of South Assam.

Acinetobacter johnsonii, generally known as a pathogenic bacterium have been found to occur as a free-living heterotrophic nitrogen fixer in the nonrhizosphere region of rice grown in acidic flooded rice fields of South Assam.

Burkholderia caribensis and Gluconacetobacter liquefaciens strains were reported as free-living or plant associated diazotrophs as they were isolated from the rhizosphere and nonrhizosphere region of cultivated rice varieties.

Azotobacter chroococcum, Azospirillum amazonense, Burkholderia caribensis, Gluconacetobacter liquefaciens and Bacillus polymyxa were found in higher number at increased soil pH in late autumn and winter seasons, whereas Beijerinckia indica, Acinetobacter johnsonii, Derxia gummosa, and Pseudomonas fluorescence have shown higher population at low soil pH in late summer and early autumn season in the rice fields.

The 16S rDNA nucleotide sequences as determined in this study for six selected strains of diazotrophs isolated from the rice agro-ecosystems on the basis of their N_2-fixing potential were deposited in NCBI GenBank. The six strains viz., Burkholderia caribensis SDSA-110/1, Acinetobacter johnsonii SDSA-119/1, Gluconacetobacter liquefaciens SDSA-128/1, Azospirillum amazonense SDSA-114/1, Beijerinckia indica SDSA-130/2 and Azotobacter chroococcum SDSA-112/1 have been assigned NCBI GenBank accession numbers GU372342, GU372343, GU372344, GU372345, GU372346, and GU372347 respectively.

Apart from diazotrophy the above strains could be exploited as microbial inoculants since they possess other PGP traits, in particular, IAA production, P-solubilization, and ACC deaminase activity. Azotobacter chroococcum, Azospirillum amazonense, Burkholderia caribensis and Gluconacetobacter liquefaciens are efficient auxin producers as well as N_2-fixers. Azotobacter chroococcum, Azospirillum amazonense and Gluconacetobacter liquefaciens strains exhibited P-solubilization capacity. Gluconacetobacter liquefaciens tested positive for Zn-solubilization. Burkholderia caribensis and Gluconacetobacter liquefaciens strains were also found to oxidize S and tested positive for cellulase and pectinase production.
Inoculation of rice with these indigenous diazotroph strains improved plant height, shoot length, root length, number of tillers/plant, plant fresh weight, weight of 100 grains, dry biomass/plant, grain yield, chlorophyll content of leaves, nitrogen content of shoot, protein content of grains and root NR activity by 5-41%, 4-37%, 2-28%, 4-43%, 5-30%, 7-30%, 11-49%, 5-35%, 9-46%, 8-47%, 8-37%, and 8-27% respectively over uninoculated control plants in both autumn and winter season. These diazotroph strains also enhanced the activity of root nitrogen assimilatory enzymes (GS, GOGAT and GDH). *Azotobacter chroococcum* was most efficient in improving the above biological parameters of winter (sali/kharif) rice whereas *Beijerinckia indica* inoculation was most effective in improving the growth and yield of autumn (ahu/pre-kharif) rice.

Inoculation of N2-fixing bacterial strains with different carrier materials such as charcoal, lignite, cured compost and vermicompost resulted significant improvement in plant height, N-uptake, dry biomass, grain yield, protein and oil content of field grown rice over control (inoculated with bacterial suspension without any carrier) plants. Vermicompost was the most efficient carrier for *Azotobacter chroococcum, Azospirillum amazonense, Burkholderia caribensis* and *Gluconacetobacter liquefaciens* in terms of survival of inoculated bacteria and improving rice growth and yield significantly. Charcoal was better carrier for *Beijerinckia indica* and *Acinetobacter johnsonii* in sustaining higher cfu count of these bacteria for a longer period and consequently, charcoal based inoculant of these bacteria improved rice growth and yield significantly.

The findings of the research work have suggested the importance of isolating more indigenous strains of diazotrophs from the rice agro-ecosystems of South Assam and study their effect on N2-nutrition of rice in order to simulate best microbial inoculant for rice cropping in this zone. This study further with respect to mapping of the nitrogen fixing (nif) genes of these diazotrophs and transfer into rice genome might be beneficial in achieving N-autotrophy by cultivated rice genotypes that would help farmers overcome the nitrogen nutrition limitations and increase the productivity, reducing dependency on chemical N fertilizers.