Rice is one of the most important cereal crops in the World cultivated throughout the five continents from tropical to temperate zones. Rice is the major staple food of nearly half of the World's population. Asia is responsible for more than 90% of total rice production around the globe. The alluvial Barak Valley plain, comprising three districts of South Assam, namely, Cachar, Karimganj, and Hailakandi is situated between longitude 92.15\(^\circ\) and 93.15\(^\circ\) East, and latitude 24.8\(^\circ\) and 25.8\(^\circ\) North, covering an area of 6,922 sq. km of land accounting 9% of total rice cropping area to share 12% of total rice production in the state of Assam. Autumn (ahu) rice as monocrop or autumn rice followed by winter (sali) rice is the important cropping pattern in this zone. Rainfall is the most important weather element for successful cultivation of rice and South Assam zone experiences a heavy annual rainfall over 3000mm. Rice being a tropical and sub-tropical plant, requires a fairly high temperature, ranging from 20\(^\circ\) to 40\(^\circ\)C. The optimum temperature of 30\(^\circ\)C during day time and 20\(^\circ\)C during night time seems to be more favourable for the development and growth of rice crop (Anonymous, 2009). The yield of rice is influenced by the solar radiation particularly during the last 35 to 45 days of its ripening period. The effect of solar radiation is more profound where water, temperature and nitrogenous nutrients are not limiting factors. Bright sunshine with low temperature during ripening period of the crop helps in the development of carbohydrates in the grains (Anonymous, 2005). All these climatic conditions required for better rice growth and yield are met in the Barak Valley alluvial plains of Assam and consequently, rice has been grown as the major cash crop in this area. Among the three rice cropping seasons viz., summer, autumn and winter, the higher yield of winter rice in Barak Valley zone over the years might be due to optimum rainfall (2000mm), temperature range (20-30\(^\circ\)C) and bright sunshine, climatic conditions best suited for better growth and yield of rice are available in this season. Winter (kharif) rice dominates in both area and production, followed by autumn (pre-kharif) rice and summer (rabi) rice in Southern Assam. In this zone, four types of rice ecosystems are prevalent, namely, rainfed upland, rainfed lowland, deep water flood prone lowland and hilly tracts and the largest share is of rainfed lowland. Rice-rice cropping pattern is being practiced in South Assam due to tropical warm humid climate with heavy annual rainfall.

Under wet or lowland rice cultivation, the land is ploughed thoroughly and puddled with 3-5 cm of standing water in the field. The optimum depth of puddling is found to be around 15 cm in the clay and clay-loam types of soil. The primary objective is to obtain a soft seedbed for the seedlings to establish themselves faster, to minimize the leaching losses of nutrients and thereby increasing the availability of nutrients and minimizing the weed problem. The land is leveled after puddling to
facilitate a uniform distribution of water and soil nutrients; and rice seedling are transplanted after a week or so. The rice agro-ecosystems of South Assam are rich in organic matter and nitrogen but due to heavy rainfall in summer months the available N is leached out which results poor availability of N for rice cultivation. Thus, it would be better if endophytic and/or free-living/associative nitrogen fixing bacteria (diazotrophs) of rice agro- ecosystems are used as inoculant in rice fields resulting in better availability of N and other minerals to the growing crop. The higher cfu count of bacteria over fungi and actinomycetes in the rice fields of South Assam explains the probable use of indigenous free-living/associative plant growth promoting diazotrophic bacteria as efficient plant inoculant for sustainable rice farming with better yield and gradual eradication of chemical fertilizer use. Thus, the rice varieties grown in flooded lowlands of South Assam are likely to harbour unique populations of N₂-fixing or plant-growth-promoting bacteria, because they are very well adapted to acid soils and often grow under low-input conditions (Kelemu et al., 2011).

The major toll of rice production in South Assam is in the winter (sali) season. Winter rice contributes to 80% of annual rice yield of Barak Valley zone. The enhanced rice yield in winter season might be due to the optimum ecophysiological condition of agro-ecosystems to support a higher microbial population specially N₂-fixing and/or PGP bacteria in and around rhizosphere region. The preference of winter (sali) rice in the rice agro-ecosystems of Barak Valley zone might be due to the fact that long term flooding of lowland rice fields during June to August each year affects maturity of autumn (ahu) rice and rainfed fields suffers from water scarcity during December to March at the time of summer (boro) rice cultivation due to lack of irrigation facility. The reasons for maximum production of winter rice are its high yield potential per unit land, higher area coverage, and preference of the crop by the farmers due to suitable agro-ecological condition of winter season.

The higher microbial count in lowland rice ecosystems might also be due to lower availability of nutrients under heavy rainfall condition resulting flooding of rice fields and higher organic C content followed by moisture content of rainfed lowland which is in accordance with the findings of Ge et al., (2010) on seasonal variation of soil microbial count in rice fields under long term application of organic and inorganic fertilizers. The higher organic C and N content of shallow water lowlands might be the reason responsible for higher microbial count in this particular habitat and this is in conformity with the findings of Ge et al., (2010). The root exudates and physico-chemical characteristics of rice field soils might be more favourable to the bacterial growth and hence bacterial population dominated the actinomycetes and fungal population. Mutualistic root microorganisms such as PGPR and diazotrophs can improve plant fitness to diverse agro-ecological environments (Sahai et al., 2011).
The role of chemical N fertilizers in the green revolution, which more than tripled the rice production in India, is beyond dispute. Over the past four decades, farmers have become increasingly dependent on chemical sources of N for obtaining high grain yields. Nevertheless, a large number of farmers still use very little or no N fertilizer because of its non-availability when needed, lack of finances, and poor yield response due to adverse conditions (Ladha et al., 1997). The use of N fertilizers though, has increased with time; the yields have often remained constant, implying a decline in yield response to N (Ladha et al., 1998). Currently, 10 ~ 12 million tonnes of chemical N fertilizers per year are used and in the next decade, it is expected that this figure would increase to 20 ~ 24 million tones (IRRI, 1993). The major concerns about chemical N fertilizers are the resource availability and sustainability, which are seriously threatened by possible increase in oil price and the estimation that current oil resources would last only for about the next 50 years (Bockman, 1997). Currently, the level of recovery efficiency for N fertilizers is only 34-40%, leading to loss of 50-70% applied chemical N fertilizers through leaching and runoff, denitrification and ammonia volatilization, because rice is grown under an environment conducive for N losses (Ladha et al., 1997). These losses lead to environmental pollution, greenhouse effect and even destruction of stratospheric ozone layer. Thus, chemical N fertilizers not only deplete non-renewable resources, but also pose human and environmental hazards. As the fossil fuel prices are not likely to remain at their present low levels for more than a decade, with ever increasing demand for food, fibre and energy from the growing human population of the tropics, high dependence on and continuous use of chemical N fertilizers may not be a sustainable option. Increases in the production cost, and the hazardous nature of chemical fertilizers for the environment has led to a resurgence of interest in the use of biofertilizers for enhanced environmental sustainability, lower cost production and good crop yields. The farmers engaged in rice farming in South Assam are mostly poor and few of them have their own farmland. The average yearly income does not support the farmers to use costly chemical fertilizer input in rice farming and nonetheless the heavy annual rainfall leaches the applied N fertilizer from the rice root zone. Therefore, the use of chemical fertilizers is not desirable from the point of production cost, yield and environmental quality in the flooded rice ecosystems of South Assam. The use of biofertilizers in rice cropping is very rare in this area which might be due to lack of availability when needed or availability after expiry and poor awareness among agricultural mass about the benefits and protocol of using biofertilizers. Thus, it is important to set up biofertilizer production units, creation of mass awareness about biofertilizers, conducting training and demonstration on biofertilizer use, isolation and identification of indigenous biofertilizer strains, formulation of higher shelf life products based on indigenous strains suited to particular environment and financial assistance to farmers in using biofertilizers might be some effective measures that can be taken to boost the production under tropical
flooded rice ecosystems of South Assam in order to achieve sustainable agriculture and economic
t betterment of farming community. According to Ishizuka (1992), total world BNF is 17.2 x 10^7 t/year.
This figure is three times of those of industrially or other ways of nitrogen fixation. The world
population increased day by day but the expansion of land is limited. Moreover, almost half of the
world’s population is consuming rice (*Oryza sativa* L.) as the primary food grain, making it the most
 important food crop currently produced (Klipp *et al.*, 2004). Hence for the higher yield of rice for over
population, people of the world use expensive nitrogenous fertilizers. These are used to fulfill the
nitrogen demand of rice that can be overcome partially by using biofertilizers when they are
scientifically applied. Microbial fertilizer is important in crop farming systems because it is an
inexpensive source of nitrogen for the higher yields of crops. This process diminishes the need for
 expensive chemical fertilizer. Thus, the extensive use of microbial fertilizer would provide economic
benefits to farmers, improve the socio-economic condition of people and preserve natural resources.

BNF, a process of harnessing atmospheric elemental nitrogen involving intricate interactions
 between associative diazotrophic bacteria and higher plants can help ensure that the supply of nutrients
 contributing to optimized yield is maintained. Free-living prokaryotes with the ability to fix
atmospheric dinitrogen (diazotrophs) are ubiquitous in soil, but our knowledge of their ecological
importance and their diversity remains incomplete till date. The capacity for nitrogen fixation is
widespread among *Bacteria* and *Archaea* (Young, 1992). The great diversity of diazotrophs also
extends to their physiological characteristics, as N₂-fixation is performed by chemotrophs and
phototrophs and by autotrophs as well as heterotrophs (Young, 1992; Paul and Clark, 1996). In natural
ecosystems, BNF by free-living, associated, and symbiotic diazotrophs is the most important source of
N (Paul and Clark, 1996). The estimated contribution of free-living N₂-fixing prokaryotes to the N
input of soil ranges from 0-60 kg/ha/year (Kennedy and Islam, 2001; Paul and Clark, 1996). The
contribution of asymbiotic relative to symbiotic N₂-fixation varies greatly, but in some terrestrial
ecosystems asymbiotic N₂-fixation may be the dominant source (Paul and Clark, 1996). Diazotrophic
bacteria due to their ability to convert N₂ to ammonia, which can be used by the plant, put themselves
in a good position to promote plant growth, because of their competitive advantage in a C-rich and N-
poor environment. The diazotrophic bacteria can well be placed under PGPR, a group of bacteria that
display beneficial effects on plant growth and yield (Dobbelaere *et al.*, 2003), since besides fixing N₂,
they also promote plant growth by other mechanisms. Diazotrophic bacterial associations with plants
besides reducing the use of nitrogen-fertilizer (Dawe, 2000), also increases the efficiency of applied
fertilizer (Okon and Labandera-Gonzolez, 1994). Though the magnitude of BNF from biofertilizer may
account for a fraction of total crop N requirement the effects of reducing losses from an ecosystem
may be equivalent to a much more significant contribution to the N economy of crop production:
(Kennedy et al., 2004). In order to exploit the full potential of the diazotrophic PGP bacterial strains, it is important to isolate those indigenous bacteria that are well adapted to the environmental conditions to utilize them as inoculant strains (Soares et al., 2006). Isolation, screening and selection of efficient PGP bacterial inoculants are important steps to optimize high crop yields and improve the sustainability of the crop systems (Roesch et al., 2007). It is plausible that the increased activity of heterotrophic bacteria might help to replace the input of N that was originally derived from cyanobacterial N fixation in rice fields (Santruckova et al., 2010). After eutrophication, however, the growth of macrophytes often increases, replacing cyanobacteria, and thus reducing autotrophic N fixation of rice fields. In our study, diazotrophic isolates were isolated from rhizosphere and nonrhizosphere region of field grown rice varieties from tropical rainfed acidic lowlands of South Assam, the aim was to screen and identify the most efficient diazotrophic bacteria that are plant growth promoting.

Nine genera of indigenous culturable diazotrophs were isolated from the acid stress rice agro-ecosystems of South Assam viz., Azotobacter, Azospirillum, Beijerinckia, Burkholderia, Gluconacetobacter, Acinetobacter, Derxia, Bacillus and Pseudomonas; and were identified by cultural, micro-morphological, biochemical and physiological characteristics following Bergey’s Manual of Determinative Bacteriology. Azotobacter population was highest followed by Azospirillum and Burkholderia which are the dominant diazotroph strains in the rice rhizosphere soils of South Assam. The cell count of Gluconacetobacter, Beijerinckia and Derxia was in the medium range in the rice fields which contributes to the indigenous diazotroph diversity of South Assam. The population of Bacillus, Pseudomonas and Acinetobacter was lower in comparison to other strains signifying their less potential to supply nitrogen needs of rice. Genera of N$_2$-fixing bacteria isolated from rice rhizosphere include Agromonas, Alcaligenes, Aquaspirillum, Azospirillum, Beijerinckia, Citrobacter, Enterobacter, Flavobacterium, Klebsiella and Pseudomonas (Roger and Watanabe, 1986). Strains most frequently isolated using exudates of rice seedlings as C source were Enterobacteriaceae, Azospirillum spp. and Pseudomonas paucimobilis (Bally et al., 1983). Occurrence of diazotrophic bacteria like H. seropedicae in association with plants of the family Poaceae, especially maize, rice, sugarcane, sorghum and wheat have been widely reported (Baldotto et al., 2011). Rice grows in flooded condition during part or all of the cropping period in about 88% of rice land. Flooding changes the chemical and microbiological property, and nutrient supplying capacity of soil. It leads to the differentiation of a range of macro- and micro-environments differing by their redox potential, physical properties, light status, and nutrient sources for the microflora. As a result, all kinds of N$_2$-fixing organisms can find conditions favourable for their growth in rice fields. They include (1)
indigenous organisms like heterotrophic aerobic, micro-aerophilic and anaerobic bacteria in soil and associated with rice, photosynthetic bacteria, and cyanobacteria; and (2) introduced green manures like *Azolla* and legumes. Traditional wetland rice cultivation has been extremely sustainable because BNF has permitted a moderate but stable yield to be maintained for thousands of years without N fertilizer addition and without deterioration of the environment. Bacteria infection of rice root tissues occur through the cavities caused by the disruption of epidermal cells during the emergence of lateral roots and the endophytic establishment by the colonization of intercellular spaces of the cortical parenchyma (Baldotto *et al.*, 2011).

In our study, among the nine identified indigenous strains of diazotroph, *Azotobacter*, *Beijerinckia*, *Burkholderia*, *Gluconacetobacter*, *Acinetobacter*, *Derxia*, *Bacillus* and *Pseudomonas* are aerobic heterotrophic bacteria and *Azospirillum* is heterotrophic micro-aerophilic bacteria. All these strains are free-living or plant associated diazotrophs as they were isolated from rhizosphere and nonrhizosphere zone of cultivated rice.

Occurrence of different genera of heterotrophic free-living aerobic diazotrophs like *Azotobacter chroococcum*, *Beijerinckia indica* and *Derxia gummosa* in the rhizosphere of rice grown in acidic soils was reported by many workers (Rao *et al.*, 1998; Xie *et al.*, 2003; Kennedy *et al.*, 2004) over the years was re-established by the findings of present investigation. The occurrence of N$_2$-fixing *Burkholderia*, *Gluconacetobacter* and *Acinetobacter* strains 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. N$_2$-fixing bacteria belonging to the genera *Azospirillum*, *Burkholderia* and *Gluconacetobacter* were isolated from rhizosphere soil of Korean rice varieties (Kang, 2006) and the isolation of these strains from rhizosphere of rice varieties grown in South Assam, India have confirmed the occurrence of these strains in rice agro-ecological habitat. In present study, report on the occurrence of *Acinetobacter* strain in the vicinity of rice rhizosphere in acidic rainfed flooded low lying agro-ecosystems of South Assam is a new finding since earlier study have reported this strain as plant and animal pathogen except in one or two cases it was reported from the rhizosphere of agricultural crop like wheat. In vitro characterization of *Acinetobacter* isolates revealed that majority of these bacteria exhibited PGP traits such as nitrogen fixation, siderophore production and mineral solubilization (Sachdev *et al.*, 2010).

The number of the isolated strains in rice root zone at maturity stage in most of the sampling sites was over $10^6$ cfu g$^{-1}$ soil which might be due to the fact that the number of N$_2$ fixers is strongly governed by soil organic matter content (Xie *et al.*, 2003) and rice agro-ecosystem soils of South Assam is rich in organic matter. The population of *Azotobacter* was highest followed by *Azospirillum* in the rice field soils of South Assam. The population of *Beijerinckia*, *Burkholderia*,
Gluconacetobacter and Acinetobacter was of medium range and that of Derxia, Bacillus and Pseudomonas was lowest.

The reason for comparatively higher population of Azotobacter, Azospirillum, Beijerinckia, Burkholderia, Gluconacetobacter and Acinetobacter might be their high N₂-fixing capacity under N-deficient rice fields as nitrogen limiting condition for microbial growth in the rhizosphere gives nitrogen-fixing microorganisms (diazotrophs) a potential advantage for accumulating in higher number (Kuiper et al., 2002). Seasonal variation of diazotroph count in the wetland rice fields is mainly due to variation of soil pH, organic C and N content, moisture availability and rice variety grown (Thakuria et al., 2009). Azotobacter, Azospirillum, Burkholderia, Gluconacetobacter and Bacillus has shown higher cell count in sali (winter) season whereas Beijerinckia, Derxia, Acinetobacter and Pseudomonas have higher number in autumn (ahu) season in the lowland rice fields of South Assam.

The rhizosphere of winter rice at tillering and panicle formation stages was better in terms of nutrient availability and ecophysiological condition to support higher population of Azotobacter, Azospirillum, Burkholderia, Gluconacetobacter and Bacillus. Likewise, the rhizosphere of autumn rice at tillering and panicle formation stages was best for the growth of Beijerinckia, Acinetobacter, Derxia and Pseudomonas due to better availability of suitable C and energy sources and ecophysiological condition. The diazotrophic bacteria of wetland rice varieties differed at different plant growth stages. Their population increases with plant age and were maximized at heading stage (Barraquio et al., 1997; Watanabe et al., 1979).

The heavy annual rainfall lowers the soil pH initiating acid stress to the native diazotrophs of rice field and the acidity of soil alters the population of diazotrophs. The rhizosphere population of Azotobacter, Azospirillum, Burkholderia, Gluconacetobacter and Bacillus showed positive correlation to soil pH accounting higher number at late autumn and winter cropping season in the cultivated rice fields of South Assam. The cfu count of Beijerinckia, Acinetobacter, Derxia and Pseudomonas is negatively correlated to soil pH confirming the higher population count of these strains in late summer and autumn season in the rice fields. 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. In the present study, it was evident that at increased soil pH in late autumn and winter season, the strains of Azotobacter, Azospirillum, Burkholderia, Gluconacetobacter and Bacillus occurred in higher number whereas the strains of Beijerinckia, Acinetobacter, Derxia and Pseudomonas showed higher population at low soil pH in late summer and early autumn season in the rice fields. Among the isolated diazotrophs, Beijerinckia, Acinetobacter and Derxia were more acid tolerant and may be effective in supplying N₂-nutrition to tropical acidic rice field soils.
Although diazotrophs rarely comprised a dominant fraction of the total rhizosphere population (Gaskins et al., 1985), rhizosphere soil frequently exhibits higher diazotroph activity as compared with bulk soil (James, 2000; Jones et al., 2003). The higher number of diazotrophs at low soil N availability is due their selective advantage over other microorganisms to fix N\(_2\) and colonize vigorously in the root region of rice (Hutsch et al., 2002). The more the N\(_2\)-fixing capacity of a diazotroph strain the more was the reduction in its population size with the rise of soil N-level and it was observed in the present investigation that the population of *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Burkholderia*, *Gluconacetobacter* and *Acinetobacter* decreases sharply at the harvesting stage of the rice crop envisaged by perfect negative correlation with N-level in rhizosphere soil.

In the present study, it was observed that the nitrogenase activity range (114.80-413.15 nM C\(_2\)H\(_4\) h\(^{-1}\) ml\(^{-1}\) culture) of isolated diazotroph strains falls within that of rhizospheric bacteria as detected by Naureen et al., (2005).

All the isolated strains were found to reduce acetylene to ethylene and thus, have potential to be used as indigenous biofertilizer inoculant for rice cropping to reduce the production cost and to achieve sustainable rice farming. The present study revealed that the acidic rice field soils of South Assam harbour good number of *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Burkholderia*, *Gluconacetobacter* and *Acinetobacter* as the major diazotrophs with higher frequency and relative abundance showing potential for N\(_2\) fixation that can be used as biofertilizer for growing rice.

Among the isolated diazotroph strains, *Azospirillum amazonense*, *Gluconacetobacter liquefaciens*, *Beijerinckia indica* and *Derxia gummosa* belonged to the class \(\alpha\)-proteobacteria; *Burkholderia caribensis* to the class \(\beta\)-proteobacteria; *Azotobacter chroococcum*, *Acinetobacter johnsonii* and *Pseudomonas fluorescense* to the class \(\gamma\)-proteobacteria and *Bacillus polymyxa* belonging to the class bacilli. The species identification was done on the basis of biochemical and physiological study according to Bergey’s Manual of Systematic Bacteriology (Krieg and Holt 1984) and Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994). The strains of *Bacillus polymyxa* are known for their beneficial effect on plant growth by phosphate solubilization and nitrogen fixation in the rhizosphere zone (Kole and Hajra, 1998). Occurrence of *Azotobacter chroococcum*, *Azospirillum amazonense*, *Beijerinckia indica*, *Derxia gummosa*, *Bacillus polymyxa* and *Pseudomonas fluorescense* as indigenous nitrogen fixing diazotroph strains in the acidic rice agro-ecosystems of South Assam have been reported earlier by Deb Roy et al., (2009a). The species *Derxia gummosa* also showed nitrogenase activity when associated with sorghum, millet, etc. (Rao and Dart, 1979). N\(_2\)-fixing *Pseudomonas fluorescense* strain was isolated from the rhizosphere of rice and several other crops (Pal et al., 2000). The findings of the present study are in conformity with that of Torres et al., (2000). The diversity analysis of diazotrophic bacteria from the rice ecosystems of South
Assam revealed that bacterial isolates were from the class α-, β- and γ-Proteobacteria, and phylum Firmicutes which is in conformity with the findings of Addison et al., (2010). Study on the nitrogen-fixing part of the microbial community around rice root region has also shown that the community is dominated by γ-Proteobacteria (Addison et al., 2010).

The trait of associative nitrogen fixing ability is being lost in modern high yielding rice varieties, which do respond well to chemical fertilizer application and retain very less number of associative N₂-fixing bacteria in root region. It is, therefore, important to characterize the strains of indigenous diazotrophs associated with the rice agro-ecosystems of any rice growing area and screen the efficient strain for inoculation in rice seedlings to lessen the application of chemical fertilizer in long run.

Based on their ARA and other PGP traits, six strains namely, *A. chroococcum*, *A. amazonense*, *B. indica*, *B. caribensis*, *G. liquefaciens* and *A. johnsonii* were selected for molecular study. They were gram-negative rods or coccii and able to fix atmospheric dinitrogen aerobically or micro-aerobically. Growth on selective media and ARA measurement was used for the purpose of isolation and selection. Previous investigations have proven these techniques to be simple, easy and economical (Dobereiner and Day, 1976; Dobbelaere et al., 2003). Sequence comparison of RNA genes of the ribosomal small subunit, 16S rDNA, is a powerful tool for phylogenetic analysis of bacterial species (Woese, 1987). Denaturing gradient gel electrophoresis (DGGE) and quantitative PCR (qPCR) are being used to analyze *nifH* and 16S rRNA genes in order to study free-living diazotrophs and the total bacterial community, respectively (Orr et al., 2011). This work presents nearly full-length primary structures of the 16S rDNA molecule of six strains of diazotroph. The sequences were obtained by semi-automated solid-phase rDNA sequencing (Wahlberg et al., 1992). The 16S rDNA gene sequence revealed the taxonomic hierarchy and molecular characterization of the strains. Direct solid-phase DNA sequencing resulted in an unambiguous determination of almost complete (>95 %) sequences of the 16S rDNA genes from the six strains of six recognized species of phylum Proteobacteria. Based on 16S rDNA sequence analysis the six selected strains were found to be the members of genera *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Burkholderia*, *Gluconacetobacter*, and *Acinetobacter* showing 99 to 100% sequence similarity.

The BLASTN program (Altschul et al., 1997) was used to search nucleotide database for similar sequences in NCBI database which showed that the isolates *A. chroococcum*, *B. indica* and *A. johnsonii* had a high similarity to members of the class γ-proteobacteria. *A. amazonense* and *G. liquefaciens* isolates showed similarity to members of the class α-proteobacteria. Likewise, *B.
The isolate 

\textit{caribensis} isolate revealed high similarity to members of the class \(\beta\)-proteobacteria. On analysis of distance matrix, similarity matrix and phylogenetic tree, it was evident that sample 1(SDSA-I12/1) has high similarity to the members of the family Pseudomonadaceae. The highest similarity of the isolate in16S rDNA sequence was with \textit{A. chroococcum} (98.6%) followed by \textit{Pseudomonas} (97.2%). The isolate SDSA-I30/2 have 16S rDNA sequence similarity with \textit{B. indica} (97.1%) followed by \textit{Pseudomonas} (95.2%). The isolate SDSA-I19/1 showed 16S rDNA sequence resemblance to \textit{A. johnsonii} (99.2%) of the family Moraxellaceae followed by \textit{Methyllobacterium}. The isolate SDSA-I14/1 showed 16S rDNA similarity to \textit{A. amzonense} (99.2%) of the family Rhodospirillaceae followed by \textit{Magnetobacterium} (95.1%). The isolate SDSA-I28/1 could be distinguished from rest of the genera of acetic acid bacteria at the molecular level. The isolate showed 16S rDNA similarity to \textit{G. liquefaciens} (99.1%) of the family Acetobacteraceae. The isolate SDSA-I10/1 have highest 16S rDNA similarity to the members of the family Burkholderiaceae and is in the same clade of \textit{B. caribensis} (99.3%). On the basis of phylogenetic analysis of 16S rRNA sequences, most of the nitrogen fixing bacteria were found to be members of the class \(\gamma\)-Proteobacteria and this was dominated by two genera \textit{Pseudomonas} and \textit{Aeromonas}. The second largest group is the Firmicutes followed by smaller proportions of clone matches to \(\beta\)- and \(\alpha\)-Proteobacteria. The 16S rDNA gene clones are heavily dominated by Gram-negative bacteria with a total of 194 out of 216 clones and only 22 out of 216 representing Gram-positive bacteria (Addison \textit{et al}., 2010). Based on sequence analysis of nifH gene two species of \textit{Frankia} (a soil-inhabiting N\(_2\)-fixing bacterium), one of \(\beta\)-proteobacteria, three strains of the genus \textit{Azotobacter}, nine strains of unculturable diazotrophs and two species from the genus \textit{Clostridium} have been identified from \textit{Brachiaria} hybrid CIAT 36062, a forage grasses, of African origin. DNA sequence analysis of these diazotrophs demonstrated that the \textit{nifH} gene sequences were highly similar to those of \textit{Klebsiella pneumoniae} and other N\(_2\)-fixing organisms and that the \textit{nif} genes had consensus sequences identical to those of other N\(_2\)-fixing bacteria (Kelemu \textit{et al}., 2011).

The 16S rDNA gene sequences of diazotrophs isolated from the rice agro-ecosystems of South Assam were deposited in NCBI GenBank. \textit{B. caribensis} SDSA-I10/1, \textit{A. johnsonii} SDSA-I19/1, \textit{G. liquefaciens} SDSA-I28/1, \textit{A. amazonense} SDSA-I14/1, \textit{B. indica} SDSA-I30/2 and \textit{A. chroococcum} SDSA-I12/1 have been assigned NCBI GenBank accession number GU372342, GU372343, GU372344, GU372345, GU372346, and GU372347 respectively. Culture-independent molecular approaches based on 16S rRNA gene analysis such as PCR amplification of 16S rDNAs, amplified ribosomal DNA restriction analysis (ARDRA), denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) have been successfully used for bacterial community analysis in a great variety of environments, including soil ecosystems, marine, rhizosphere,
food, and human intestine to overcome the limitations of culture-dependent approaches (Sun et al., 2008).

In recent decades, increasing evidences indicated that, besides increased nutrient uptake, the synthesis and export of phytohormones by microorganisms may also play an important role in plant growth promotion which is also true for diazotrophs (Dobbelaere et al., 2003; Roesch et al., 2007). The selected diazotrophs from rice ecosystems of South Assam in this study were equally efficient in IAA production as some of the diazotrophic bacteria isolated from the maize collected from five regions within the Southern State of Rio Grande do Sul in Brazil (Roesch et al., 2007). In the present work, it was observed that *A. chroococcum* (SDSA-I12/2) produced maximum amount of IAA and tested positive for ACC deaminase activity and *B. caribensis* (SDSA-I10/1) has highest activity of ACC deaminase as well as tested positive for siderophore production. The presence of *B. caribensis* has not been previously noted in rice ecosystems; however, other spp. of this genus has been documented to be potential PGPB in certain plants including rice (Govindarajan et al., 2008). *Azotobacter, Azospirillum, Burkholderia* and *Gluconacetobacter* strains showed relatively high IAA production compared to *Beijerinckia* and *Acinetobacter* strains. This finding revealed that *Azotobacter, Azospirillum, Burkholderia* and *Gluconacetobacter* are efficient auxin producers as well as N$_2$-fixers. The present study confirmed the wide distribution of ACC deaminase activity in diverse diazotrophic bacterial genera, in accordance with previous studies (Belimov et al., 2001). Furthermore, the *Burkholderia* and *Acinetobacter* strains evaluated in the present study have higher ACC deaminase activity than the other strains. Bacteria containing ACC deaminase bind to roots and/or seed coats and stimulate root elongation by lowering the ethylene level in plants (Li et al., 2000). It is not essential that a single PGPR should possess all the PGP characteristics and can promote plant growth even using a single mechanism. The results of this study confirmed the wide distribution of ACC deaminase activity in different bacterial genera concurrent to previous studies (Belimov et al., 2001; Shaharoona et al., 2007; Poonguzhali et al., 2008). The presence of ACC deaminase is a positive factor for all strains, since a recent report suggested that the ACC deaminase of the *Pseudomonas brassicacearum* Am3 could promote plant growth in tomato by masking the phytopathogenic properties of this bacterium (Belimov et al., 2007). Phosphorus and Zn are two important plant nutrients, and the beneficial role of PGPB in maintaining adequate levels of these mineral nutrients in crop production has been previously reported (Rodriguez and Fraga, 1999; Saravanan et al., 2007). *A. chroococcum, A. amazonense* and *G. liquefaciens* strains exhibited P solubilization capacity. *B. caribensis* and *G. liquefaciens* were also found to oxidize S, which would enhance the production of sulfates and make them available for plant growth (Wainright, 1984). Sulfur reducing bacterium like *Gluconacetobacter* was an important component of N$_2$-fixation, contributing from 20 to 53% to the overall N$_2$-fixation.
Bacterial siderophores are an important class of compounds that enhance plant growth and protect plant health by binding to available iron (Fe$^{3+}$) in soils (Vial et al., 2007). It was found that only *B. caribensis* strain produce siderophores. All the strains tested positive for NH$_3$ production, but were negative for HCN production. The positive test for cellulase and pectinase production by *B. caribensis* and *G. liquefaciens* strains indicated that they are able to infect rice root and form some sort of endophytic association.

PGP activity was assessed by the ability of synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and lessening or preventing the plants from diseases (Hayat et al., 2010).

Among the six strains, the highest nitrogenase activity was shown by *A. chroococcum* and the nitrogenase activity range of the strains was 305.2-484.2 nM C$_2$H$_4$ h$^{-1}$ mg$^{-1}$ protein which is in conformity with the findings of Naureen et al., (2005). The endophytic bacteria have an advantage over root-associated diazotrophs, as *Beijerinckia* spp. and *Azotobacter* spp., they colonise the interior rather than the surface of plants, and hence have better possibilities to exploit carbon substrates supplied by the plant (Boddey et al., 1995; Triplett, 1996). Some characteristics distinguish *Gluconacetobacter* as the high sucrose (10%) tolerance, growth and nitrogen fixation at low pH (5.0 or less) and that nitrogen fixation is only partially inhibited by NH$_4^+$, especially at high sucrose concentrations (Boddey et al., 1995). Kirchhof et al., (1997) observed a higher number of diazotrophic bacteria associated with graminaceous plants if no fertilizer was applied.

The better competence of *B. indica* over other diazotroph strains in colonizing the rhizosphere of autumn (ahu) rice is due to its higher tolerance to soil acidity and partial anaerobic condition prevailing in the rhizosphere during autumn season. The higher number of *A. chroococcum* in the rhizosphere of winter (sali) rice of Ranjit variety may be due to its selective influence. The population of *H. seropedicae* Z67 in rice plantlets have increased between 5 to 7 days after inoculation to $10^6$ and then decreased to values between $10^3$ and $10^4$ log cfu/g fresh weight. This population dynamics reflected the nonpathogenic nature of *H. seropedicae* and the capacity of the plant genotype to host the bacterium (Baldotto et al., 2011).

The GS activity was higher than GOGAT and GDH activity in plants inoculated with the diazotroph strains. Higher activity of GS and GOGAT than GDH revealed that ammonia assimilation was carried out in the rice plant cytoplasm by the coupled enzyme complex system of the two enzymes GS-GOGAT. Yuan et al., (1990) reported that GS-GOGAT cycle appeared to be the only pathway for ammonia assimilation in the developing rice grain. Ohyama and Kumazava (1980) also reported that ammonia was assimilated by GS-GOGAT system. Kush et al., (1985) reported that assimilation of ammonia formed in the process of nitrogen fixation primarily by the reaction catalysed by glutamine
synthetase (GS). In free-living diazotrophs, the nitrogenase generated ammonium is assimilated into glutamate through the glutamine synthetase/glutamate synthase pathway (Deb Roy and Deb, 2010). Since the total nitrogen content of shoot in *A. chroococcum* inoculated plants in autumn season was highest compared to other strains, the GS activity was also higher. Similarly the N-content of shoot of winter rice was higher in *A. chroococcum* inoculated plants which have shown higher activity of GS in comparison to other strains. The lowest activity of nitrogen assimilatory enzymes was shown by plants treated with mixed inoculum of all strains and the specific activities were still higher than the uninoculated control. Among the six strains, the highest GDH activity was revealed by *A. amazonense* inoculated plants in both autumn and winter crop and this may be due to higher capacity to utilize ammonia than the other strains (Deb Roy *et al.*, 2009b). The GDH activity was lower in plants inoculated with diazotroph strains than GS and GOGAT activity at all stages of growth and this fact may be attributed to the presence of low ammonia concentration in the field as because the field was left un-cropped in the previous cropping season due to flooding. Inoculation of diazotrophic bacteria increased the activity of N assimilatory enzymes in rice roots 30 days after sowing, which signifies the efficient transfer of N to plants by the inoculated diazotrophs.

The nitrogenase enzyme of diazotrophs is solely responsible for their N$_2$-fixation ability and the more the number of diazotrophs in the soil the higher is the nitrogenase activity of soil (Jha *et al.*, 2009). The higher nitrogenase activity of rhizosphere inoculated with *B. indica* and *A. chroococcum* may be one of the important factors for higher growth and yield of rice; chlorophyll$_a$, N and protein content; better N-uptake and growth and yield in autumn and winter growing seasons. The rhizosphere population of inoculated diazotrophs directly affects the nitrogenase activity of rhizosphere soil. In the present study, it was observed that higher number of inoculated diazotrophs in the rhizosphere of rice promoted higher nitrogenase activity of rhizosphere soil. *A. chroococcum*, *A. amazonense*, *B. indica*, and *B. caribensis* inoculants have shown significantly higher rhizosphere population and thus, higher nitrogenase activity of rhizosphere soil of inoculated rice seedlings. The AR activity of rhizosphere soil of diazotroph inoculated rice plants is positively correlated to the height of the plants at the harvesting stage (Adesemoye *et al.*, 2009). In the present study, it was observed that AR activity of rhizosphere soil is positively correlated to the height of diazotroph inoculated rice plants.

Treatment of rice seedlings with diazotroph strains improved the plant height, shoot length, root length, number of tillers/plant, plant fresh weight, weight of 100 grains, dry biomass/plant, chlorophyll$_a$ 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%, 9-46%, 8-47%, 8-37%, and 8-27% respectively over uninoculated control plants in autumn and winter season. Seedlings treated with diverse diazotrophic strains showed a significant increase in plant height, shoot length, root length,
number of tillers, fresh weight, grain weight, dry biomass, chlorophyll content, N and protein content, NR activity and GS, GOGAT and GDH activity compared to the control. In a similar study conducted under gnotobiotic conditions, Baldani et al. (2000) reported that the inoculation of rice with *Herbaspirillum seropedicae* enhanced plant dry weight by 71.5%. Similar results were reported by Yim et al. (2009) in diazotrophic bacteria isolated from Chinese cabbage. Combined application of biofertilizers with or without crop residue incorporation is known to improve NUE and higher yields in rice based cropping systems (Regmi et al., 2002; Yadavinder Singh et al., 2004; Reddy and Raju 2006; Pampolino et al., 2007). Inoculation experiments with the endophytic bacterium *Azospirillum* sp. strain B510, an isolate from surface sterilized stems of field-grown rice, were conducted in pots in a greenhouse, and in paddy fields in Hokkaido, Japan which revealed that B510 significantly enhanced the growth of newly generated leaves and shoot biomass under greenhouse conditions. When rice seedlings were treated with $1 \times 10^8$ cfu ml$^{-1}$ and transplanted to paddy fields, the tiller number and seed yield significantly increased (Isawa et al., 2010). In a field experiment, it was observed that single inoculation of free-living and associative diazotroph strains like *Azotobacter chroococcum*, *Azospirillum amazonense* and *Beijerinckia indica* improved plant height, dry biomass, number of tillers, weight of grains, chlorophyll content of leaves, N-content of shoot, and protein content of grain of autumn rice by 23-29%, 17-49%, 21-29%, 20-28%, 18-38%, 24-42%, and 17-31% respectively over uninoculated control plants in acidic lowland agro-ecosystem (Deb Roy et al., 2009c).

The GS activity of diazotroph inoculated rice plants have shown a direct effect on the N-content which is due to the assimilatory function of GS in the process of N-uptake by plant roots (Adesemoye et al., 2009). The higher GS activity of roots promoted better uptake of N by treatment plants which is reflected in higher N-content of shoot. The *B. indica* and *A. chroococcum* were the two diazotroph strains which imparts higher GS activity in the roots of autumn and winter rice respectively and thus, higher N-content of shoot. The diazotrophs capable of stimulating high GDH activity of inoculated rice plants also improved the NR activity of roots for enhanced uptake of mineral N and dry biomass production.

*Azotobacter chroococcum*, *Azospirillum amazonense* and *Beijerinckia indica* are the dominant diazotroph strains of acidic lowland rice fields of tropics and these can be used as biofertilizer for cultivated rice (Deb Roy et al., 2009c). Single or dual diazotroph inoculum has been beneficial for rice growth and yield enhancement comparable to chemical fertilizers but multistrain biofertilizer inoculum has been successful only in few cases to improve yield of field grown rice (Constantino et al., 2008). Thus, it is important to select the individual members of a multistrain biofertilizer inoculum with different niche requirements to minimize the interspecific competition for enhanced PGP effects. Inoculation with diazotrophic bacteria increased the activity of N assimilatory enzymes in rice roots.
after 30 days of transplantation, which signifies the efficient transfer of N to plants by the inoculated diazotrophs.

It was observed that dual inoculation of wheat with diazotrophic PGPR increased grain yield by 41% as compared to un-inoculated control. The diazotrophic PGPR consisted of two fluorescent *Pseudomonas* strains (*Pseudomonas jessenii*, R62; *Pseudomonas synxantha*, R81), derived from wheat rhizosphere. The raw protein (nitrogen × 5.7) and mineral nutrient concentration of wheat grains (phosphorus, potassium, copper, iron, zinc, manganese) were higher after inoculation (+6% to +53%) with diazotrophic PGPR. Effects of these strains on rice and black gram yields were far less pronounced suggesting that PGPR isolated from the target crop were more efficient (Sahai *et al.*, 2011).

The improvement in the nutrient availability and fertility of soil of the experimental plot after transplantation of diazotroph inoculated rice seedlings may be attributed to the high N₂-fixing capacity of the diazotroph strains in the rhizosphere of rice coupled with several other PGP traits (Ramezanpour *et al.*, 2010).

The soil health and nutrient availability are the key components for a better agricultural productivity and treatment of rice seedlings with carrier based diazotroph inoculants is one of the measures that have been practiced recently to reclaim soil nutrient status and sustainable rice production keeping the yield intact as achieved using chemical inputs or sometimes even enhanced (Ramezanpour *et al.*, 2010). PGPR application improved soil quality as indicated by increased soil enzyme activities of alkaline and acid phosphatase, urease and dehydrogenase (Sahai *et al.*, 2011). In the present study, it was observed that single inoculums of *A. chroococcum*, *A. amazonense*, *B. indica*, *B. caribensis*, *G. liquefaciens* and *A. johnsonii* have shown significant improvement in the tested nutritional parameters of rice field soil in both autumn and winter season. As sessile organisms, plants have evolved different strategies to adapt to changes in the availability and distribution of N in soils. These strategies include mechanisms that act at different levels of biological organization from the molecular to the ecosystem level. At the molecular level, plants can adjust their capacity to acquire different forms of N in a range of concentrations by modulating the expression and function of genes in different N uptake systems. Modulation of plant growth and development, most notably changes in the root system architecture, can also greatly impact plant N acquisition in the soil. At the organism and ecosystem levels, plants establish associations with diverse microorganisms to ensure adequate nutrition and N supply (Kraiser *et al.*, 2011).

The findings of present investigation on the PGP traits of diazotrophs and their effect on the growth and yield of field grown rice in autumn and winter season suggested the role of these strains in biofertilization of rice under tropical rainfed acidic ecosystems of South Assam and Northeastern
states. It was evident that the acidic rice field soils of South Assam harbour good number of *A. chroococcum*, *A. amazonense*, *B. caribensis*, *G. liquefaciens*, *B. indica* and *A. johnsonii* strain as the major diazotrophs with higher frequency and relative abundance which have shown potential for N$_2$ fixation and can be used as biofertilizers. Studies predicted that diazotrophic bacteria help in maintaining soil fertility and N input in disturbed ecosystems (Cvijanovic et al., 2006; Kennedy et al., 2004). The obtained results are in agreement with those obtained by El-Shahat et al., (2002). Therefore, it was evident that the principle mechanism by which these diazotroph strains have benefited the plant growth was by fixing molecular nitrogen and its transfer to the plant as direct effect or production of plant growth hormones (IAA) by these bacteria which could release in the root media and affect its growth and extension positively.

Statistically significant grain yield increases due to *Azospirillum* inoculation have been reported from India and Israel (Dobereiner, 1989). But most of the cases, these trials have been done using bacterial strains from International collections. A collection of nine N$_2$ fixing bacterial strains were isolated from the rhizosphere of rice varieties cultivated in acidic rainfed lowland ecosystems of South Assam to characterize and identify indigenous nitrogen-fixing organisms of rice rhizosphere soil in order to reduce the use of chemical nitrogen fertilizers by the production of biofertilizer in this zone. Microbial interactions play very important role in sustainable agriculture through the integrated nutrient supply. It is important to lay emphasis on the application of efficient microbial inoculants in crop production (Rengel and Marschner, 2005). Various commercial mono-culture inoculants being produced in India by various government/semi-government or private agencies includes *A. rhizobia* and *B. rhizobia, Azospirillum, Azotobacter, Bacillus megaterium, Bacillus polymyxa*, mycorrhizal inoculants for forest trees and *Pseudomonas* as PGPR and biocontrol agent. Application of efficient inoculants of *Rhizobium, Mesorhizobium* and *Bradyrhizobium; Azospirillum, Azotobacter, Pseudomonas* and phosphate solubilizing microorganisms for the improvement of crop productivity and plant nutrition is well documented (Al-Taweil et al., 2009). The present study assessed charcoal, lignite, compost and vermicompost as carrier of indigenous diazotroph strains and the impact of carrier based inoculants of these strains on rice seedlings. The cfu count of bacterial cells in different inoculants revealed the high level survival of *A. chroococcim, A. amazonense, B. caribensis* and *G. liquefaciens* in vermicompost based carrier. The higher survival exhibited by these bacterial strains when vermicompost was used as carrier material can be attributed to the fact that the micro-environment of this carrier is better suited to the growth requirements of these bacteria. Earlier reports (Bashan and Holguin, 1997; Bashan et al., 1998) revealed that the energy and carbon storage in vermicompost is better used under stress conditions, such as limitations of carbon and energy, a capacity that enhanced the survival under stress conditions. *B. indica* and *A. johnsonii* have snow:
better survival in charcoal based carrier and this fact may be attributed to the fact that these strains require more carbon and moisture under stress condition which were made available by charcoal powder. Inoculation of native N$_2$-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 raw bacterial suspension) plants. The difference in the growth and yield parameters among different inoculant carriers is statistically significant at 5% significance level.

Since the triple bond of atmospheric N$_2$ is stable, the reduction of N$_2$ to ammonia during its biological fixation is an expensive process energetically. Therefore, diazotroph incidence and activity depends on the availability of energy rich carbon (C) sources. The continual input of easily available C into the rice rhizosphere sustains high activity of root associated microflora and thus, diazotrophs are closely associated with rice roots (Santruckova et al., 2010). The heterotrophic N$_2$-fixation substituted in part for autotrophic cyanobacterial N$_2$-fixation when P limitation was alleviated.

Several laboratory as well as field level application of vermicompost as carrier material for microbial inoculants have shown higher growth rate of plants, increased uptake of nutrients and increased rate of yield in crops like paddy, tomato, green gram and cow pea (Karmegam et al., 1999; Manivannan and Daniel, 2009). The present study indicated that vermicompost can very well be used as carrier material for A. chroococcum, A. amazonense, B. caribensis, and 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 water logged acidic rice fields. Likewise, the charcoal based preparation of B. indica and A. johnsonii has shown higher number of viable cells and charcoal based inoculant resulted significant improvement in growth and yield parameters of rice which suggests that charcoal can be used as efficient carrier of B. indica and A. johnsonii in biofertilizer composition of rice grown in tropical rainfed acidic lowlands. The further elucidation of their role in rice growth under tropical rainfed lowland rice ecosystems will prove these free-living/associative diazotrophs as potential microbial inoculants and a valuable partner in future rice cropping in tropical regions. The better survival and colonization of carrier based diazotroph strains in the rhizosphere of inoculated rice seedlings is one of the factors contributing to the higher grain yield and the findings of the present work supported the fact that the rhizosphere population of inoculated diazotroph strains is positively correlated to the grain yield of rice (Elkholy et al., 2010). It is assumed that the rice seedlings treated with carrier based diazotroph inoculants would have luxuriantly excreted organic acids, especially malate, useful for the proliferation of diazotroph spp.
The findings of the present investigation revealed that indigenous strains of heterotrophic free-living associative and/or endophytic diazotrophs namely, *A. chroococcum*, *A. amazonense*, *B. indica*, *B. caribensis*, *G. liquefaciens*, and *A. johnsonii* isolated from the rhizosphere and nonrhizosphere region of flooded rice fields are efficient N\(_2\)-fixers and possesses 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\(_a\) content of leaves, 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 autumn and winter rice under acidic flooded ecosystems. The carrier based inoculants of these strains were more efficient than without carrier in improving plant height, N-uptake, dry matter, grain yield, protein and lipid content of rice plants. Vermicompost was the most efficient carrier for *A. chroococcum*, *A. amazonense*, *B. caribensis*, and *G. liquefaciens* in terms of survival of inoculant bacteria and improving rice growth and yield significantly. Charcoal was the best carrier for *B. indica* and *A. johnsonii* in supporting 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 above findings supported the hypothesis that “Nitrogen fixing diazotrophs may tolerate the flooding and some bacterial strains may be efficient under anaerobic soils of rhizospheric and nonrhizospheric region of rice in fixing atmospheric nitrogen and improving the growth and yield of rice”.

However, because rice production often remains “N responsive” even when combined with rhizobial inoculation, further efforts must be exerted to reasonably eliminate the need for additional fertilizer-N inputs to obtain higher rice yield. Perhaps the solution to achieve that ultimate goal is to critically formulate microbial fertilizers containing mixed inoculant consortia that include the best-performing rhizobial strains (*Azotobacter*, *Azospirillum* and *Beijerinckia*) plus highly selected free-living N-fixers like cyanobacteria; mycorrhizae association like VAM and other PGP rhizobacteria like *Burkholderia* to eliminate the dependency on chemical fertilizers in the flooded rice fields of South Assam. It is hypothesized from present study that inoculation with carrier blended single strains of native diazotrophs significantly increased the growth and yield parameters of rice under field condition in autumn and winter season. By combining superior rhizobacterial inoculants with agricultural extension training, grain yield increased up to 47% in farmers’ fields, with an average increase of 19.5% (Yanni and Dazzo, 2010). The results of this research indicated that environment friendly rhizobial biofertilizer inoculants can be recommended with sufficient supporting data to improve agriculture economy and sustainable agro-ecosystem maintenance for increased rice grain production.
where the benefits of such biotechnology are most urgently needed, e.g., the important hunger alleviating mission of the Emergency Rice Initiative.

The most studied microbial groups responsible for BNF in flooded rice fields are free-living cyanobacteria, and symbiotic cyanobacteria associated with the fern Azolla but the present study has focused on free-living and associative heterotrophic bacteria. The results revealed that native strains of free-living and associative heterotrophic bacteria caused significant increase in growth and yield components, and grain N-content. It is generally believed that organic matter incorporation provides a better environment for the proliferation of the introduced bacteria and for BNF by improving chemical and physical properties of the soil and this fact was established by the positive yield responses of rice to vermicompost or charcoal based inoculants of native diazotroph strains. Results have confirmed under different field conditions with different rice cultivars, inoculation of rice with carrier based inoculants of novel diazotroph strains would allow a considerable decrease in the use of chemical fertilizers in rice cultivation, which is economically and environmentally important. The yield improvement may be due to other mechanisms besides nitrogen fixation. One of the other possible mechanisms could be the production of growth-promoting and growth regulating substances by these diazotrophic bacteria (Eldin and Elbanna, 2010). To our knowledge, this is the first reported evidence regarding the potential of B. caribensis, B. indica, G. liquefaciens and A. johnsonii as a biofertilizer for flooded rice under field condition. Through optimizing rice cultivation and bacterial inoculation, it is possible to obtain an increase in grain yield and a decrease in chemical N fertilizer requirement. These diazotrophic bacteria have great potential for the development for use as biofertilizers in flooded rice cultivation where chemical N fertilizers are unavailable or expensive and in rice production systems that aim to minimize the environmental impact of an intensive use of chemical N fertilizers. Therefore, efforts need to be focused towards enrichment of indigenous diazotroph populations, which are better adapted to the specific niche, through development of carrier based inoculums on a regional basis. Research programs should be oriented towards agricultural practices, including application of microbial inoculants, which would enhance the growth and proliferation of indigenous diazotroph strains.