CHAPTER 6

CONCLUSSION

6. Conclusion

The term rhizobia are used for all the bacteria that are capable of nodulation and nitrogen fixation in association with legumes. Occurrence of rhizobia in varied environments and their significance emphasizes the need for the exploration of legume plants to know the rhizobial diversity. The major challenge in exploiting the enormous potential of symbiotic rhizobia is the selection and development of efficient inoculants strains compatible to legume host. The present study provided a detail report on the phenotypic, biochemical and genotypic features of rhizobia associated with the root nodules of *L. purpureus*, *C. cajan*, *C. pallida* and *M. pudica* from different geographical locations of Assam, India. In addition the present study also establishes the plant growth promoting and stress tolerant abilities of the isolates. The study also provided the basic knowledge of phylogeny and diversity of rhizobial strains associated with the selected legume plants, as well as their use as inoculants to improve the growth of their host plants. In addition the siginificance our present study also lies in the fact that for the first time it reported the presence of standard alpha-rhizobia, beta-rhizobia and endophytic bacteria from the root nodule of both cultivated and wild leguminous plants of Assam.

In the present study a total of 120 bacterial cultures was isolated from the root nodule of the selected legume plants collected from different sites of Assam and studied for their phenotypic, biochemical and genotypic features. In phenotypic characterization, most of the isolates showed standard features of rhizobia i.e. white or off white color, round shape, 1-3 mm size colonies, gram negative staining and rod shaped cells. The phenotypic features and confirmatory test results were compared with the phenotypic features and confirmatory test results of reference strains. The results clearly indicated that the majority of the isolates showed close similarity with

rhizobia. In confirmatory tests most of the isolates form white colonies on YEMA-Cr and showed negative results of ketolactose test. Thus, based on the results of primary screening 92 probable rhizobial isolates were selected for further study.

The ability to produce enzymes to utilize various organic substrate is an important feature of rhizobia. also For determining the enzymatic abilities of selected isolates 7 biochemical tests were carried out by following the standard procedure. Upon biochemical characterization almost all the isolate showed positive results for catalase and oxidase test which clearly indicated the ability of the these isolates to produce catalase and oxidase enzyme. It was also revealed that most of the isolates were incapable of utilizing starch as a sole carbon source as they showed negative results for starch hydrolysis test. The selected isolates showed variable results of nitrate reduction, urease production, citrate utilization and gelatin hydrolysis test. Thus from the present study it can be concluded that the isolates efficiently produces different metabolic enzymes which siginificantly helps in the nodulation and nitrogen fixation process.

The present study confirmed the plant growth promoting abilities of the isolates associated with the legume plants of Assam. IAA production is believed to be important for promotion of rootelongation and root hair development. In the present study majority of the isolates were capable of IAA production and few isolates produced higher amounts of IAA than the reference strains. It was also confirmed that isolates obtained from all the 4 legumes produced good amount of IAA, in fact the best 3 isolates *viz.* SDL1, HMP1 and MKCP1 which, produced the highest amount of IAA were obtained from 3 different legume plants. Present study also confirmed phosphate solubilizing ability of the isolates. Among all the isolates investigated, 21 isolates showed higher phosphate solubilization efficiency then the reference strains

and among them 3 isolates *viz.* DCP1, IDL1 and RCP2 showed best result. In addition to IAA production and phosphate solubilization, present study also confirmed the ammonia production ability of the isolates. The results of the current study clearly indicated that these plant growth promoting isolates could be beneficial to plants, especially to minimize the excessive inputs of inorganic fertilizers in soil. Thus, these isolates could be used as an effective inoculant for the growth of their host plants in agricultural practices.

In the present study the isolates showed marked variation in the acidic pH and salt tolerance abilities. Soil pH is an important parameter for the growth of microorganisms. Slight variation in pH may have huge effects on the growth of organisms. In the current study pH 6 is found to be the most suitable pH for the growth of the isolates and all the isolates showed remarkable growth at pH 6. However a good number of isolates also survived at pH 5. On the contrary the growth of isolates was inhibited at pH 4, except 5 isolates *viz.* KACP2, GCP1, RMP2, SIMP3 and KMP2 which, showed visible growth at pH 4. Salinity influences the growth and survival of rhizobia through alterations in the permeability of the membrane and uptake of nutrients. The results of the present study revealed that the isolates can survive and showed visible growth on YEMA media incorporated with 1% and 2% NaCl. However, the survivability of isolates decreases at higher NaCl concentration and only 4 isolates viz. RCP2, KACP2, SICP2 and SMP2, survived and showed growth at 3% NaCl. The results indicated that the isolates were sensitive to high salt concentration. A major advantage of indigenous rhizobial strains is their adaptability to diverse environmental stress conditions. This kind of specific adaptation of the strains to environmental stress is used as an approach for the development of bioinoculants for their host legumes. The present study provided detailed knowledge

about the rhizobial isolates associated with legumes of Assam which can tolerate a wide range of acidic pH and salt concentration. The study also confirmed the presence of few isolates which tolerated extreme level of stress. Consideing the above findings it could be rightly concluded that the isolates which showed extreme stress tolerance abilities were highly efficient and could be useful as inoculants for host legume plants growing in a stressed environment.

Amplification of *nifH* in 29 isolates clearly indicated the ability of these isolates to synthesize nitrogenase enzyme. Likewise, successful amplification of *nodC* genes in 27 isolates indicated their ability of producing nod factors. Few endophytic bacteria failed to amplify *nodC* genes with the selected primers. Based on plant growth promoting and stress tolerance abilities 40 rhizobial isolates were selected for genotypic characterization. Genotypic characterization was carried out by 16S rDNA RFLP analysis and phylogenetic identification of the isolates was carried out by sequencing of 16S rDNA gene. The restriction pattern revealed the presence of several DNA bands which ranges in size from 100 bp to 1000 bp. Dendrogram generated from restriction pattern demonstrated a high level of genetic diversity among the isolates. RFLP analysis groups the isolates of selected legume plants into different clusters bssed on their restriction patterns. The RFLP analysis also revealed the genotypic relationship of isolates with the reference strains.

Results of 16S rDNA sequencing revealed the phylogenetic identity of the isolates. In the present study, 16 isolates (4 isolates from each legume plant) were selected for 16S rDNA gene sequencing. Sequencing and NCBI-BLAST analysis of selected isolates revealed that all the *L. purpureus* isolates belong to *Bradyrhizobium* genera. The isolates were identified and named as ***Bradyrhizobium elkanii*** **HIDL1**, ***Bradyrhizobium lablabi*** **SDL1**, ***Bradyrhizobium pachyrhizi*** **SIDL1** and

***Bradyrhizobium elkanii* DDL2**. Likewise, it was found that isolates associated with*C. cajan* were phylogenetically related to *Burkholderia, Mesorhizobium* and *Rhizobium* genera. The isolates obtained were identified and named as ***Mesorhizobium loti* ICC1**, ***Burkholderia cenocepacia* ICC3**, ***Rhizobium mayense* DCC2** and ***Burkholderia anthina* SICC1**.Sequence analysis of *C. pallida* isolates revealed that the isolates belong to *Mesorhizobium* and *Bacillus* genera.The isolates were identified and named as ***Bacillus megaterium* DCP1**, ***Mesorhizobium jarvisii* RCP2**, ***Bacillus koreensis* NCP1** and ***Mesorhizobium jarvisii* KACP2**. The results also revealed that beta-rhizobia belonging to the genera *Burkholderia* are the main symbiont associated with *M. pudica*. The isolates were identified and named as ***Burkholderia mimosarum* SMP2**, ***Burkholderia mimosarum* HMP1**, ***Burkholderia mimosarum* DMP2** and ***Mesorhizobium loti* KMP1**. These results clearly demonstrated that root nodule bacteria associated with legume plants of Assam consist of both alpha and beta-rhizobia. In addition to these usual symbiotic rhizobia, endophytic rhizobia belonging to the genera *Bacillus* were also present. Thus the results of current study enriches our knowledge about the diversity and phylogeny of rhizobia associated with the legumes of Assam.

Present study also confirmed nodulation ability of the isolates on their host plants. Except two endophytic strains all the inoculated isolates nodulated their host plants. Un-inoculated control plants were found without nodules. The isolates which form nodules on their host plants include 4 isolate of *L. purpureus*, 2 isolates of *C. cajan*, 2 isolates of *C. pallida* and 4 isolates of *M. pudica*. However, 2 non-nodulating endophytic isolates representing *Bacillus* genera were unable to form nodules on their host plant *C. pallida*. Additionally, the present study also revealed that inoculation of isolates significantly increases different vegetative parameters *viz.* shoot length, shoot

dry weight, root length of the host plant. The increase of different vegetative parameters after inoculation of isolates could be directly attributed to the beneficial effects from biological nitrogen fixation and indirectly to the plant growth promoting abilities of the isolates. Thus, from the present study it is confirmed that the rhizobial isolates associated with legume plants of Assam, helps in growth and development of their host plants and could be used as a bioinoculant in agricultural activities.

The agricultural goal of present day world is a high output of the crop in an environment friendly manner. Inoculation of efficient rhizobial strains in crop is important for sustainable agricultural development. The present study suggested the presence of diverse rhizobial strains in the root nodules of *L. purpureus, C. cajan, C. pallida* and *M. pudica* grown in Assam, India. In addition the present study confirms the presence of beta-proteobacteria in the root nodules of *M. pudica* and endophytic bacteria in the root nodules of *C. pallida* which was not reported earlier from this region. The isolates obtained from the legumes showed plant growth promoting abilities and varied level of tolerance to environmental stress. Genotypic studies of the isolates also confirm the presence of specific genes required for nitrogen fixation and nodulation. This beneficial indigenous isolates could be useful for preliminary bio-fertilizer development and thus could be used as a cheaper substitute for chemical fertilizers. In conclusion it can be said that the present study revealed new information about the rhizobia legume symbiosis ingeneral and diversity of rhizobia associated with leguminous plants of Assam in particular. The present study also suggested that there is an urgent need for growing efficient, indigenous and environment friendly rhizobia in large scale for providing its beneficial abilities to small scale farmers.