CHAPTER 5

DISCUSSION

5. Discussion

Rhizobia are very diverse at species and strain levels. Enormous diversity exists in rhizobia due to their different plant hosts and wide geographical distribution (Martinez-Romero & Caballero-Mellado, 1996; Madrzak *et al*., 1995). Due to their huge agricultural and environmental significance, a large number of works have been carried out tostudy the diversity ofindigenous rhizobia from various regions of the world. In the last few decades, many researchers from different parts of the world have studied the genetic diversity of rhizobia from different legume plants such as *Pisum sativum*, *Lablab purpureus*, *Cicer orientum*, *Cajanus cajan*, *Glycine max*, *Mimosa sp*., *Phaseolus vulgaris, Vicia sp*., (Chen *et al*., 2000; Chang *et al*., 2011; Chen *et al*., 2008; Chen *et al*., 2001; Cigdem *et al*., 2006; Dubey *et al*., 2010). The isolation and characterization of rhizobia from different leguminous plant is an interesting field of work that helps to understand the diversity and evolution of rhizobia. However, despite the huge importance of rhizobia, little is known about the diversity of native rhizobia from north eastern part of India. In particular, very little taxonomic work has been done on the symbiotic bacteria associated with legume plants growing in Assam, India. Keeping all these facts and findings in mind, the present studywas undertaken with the objectives to study the genetic diversity of plant growth promoting and stress tolerant rhizobia from root nodules of few selected leguminous plants from different geographical regions of Assam.

As per the mentioned objectives 4 leguminous plants, *Lablab purpureus*, *Cajanus cajan, Crotalaria pallida* and *Mimosa pudica* were selected for the present study. Many previous reports suggested the presence of diverse rhizobia associated with these 4 selected plants in different parts of the world (Chang *et al*., 2011; Dubey *et al*., 2010; Liu *et al*., 2007). The cultivated legumes *L. purpureus* and *C. cajan,* were cultivated and consumed by the people of Assam since ancient time. In Assam, *L. purpureus* is grown as an annual legume plant in agricultural fields in different regions. Till date, only a few rhizobial strains isolated from *L. purpureus* nodules have been studied and most of these have been identified as members of the genus *Bradyrhizobium* (Chang *et al*., 2011). In recent past, Amel *et al*. (2013) isolated rhizobial strains from root nodule of *L. purpureus* collected from Algeria based on their phenotypic features (Amel *et al*., 2013). *Cajanus cajan* or pigeon pea is also an important pulse crop and a very popular food in developing countries. India is a principal pigeon pea growing country contributing nearly 90% of total world's production. Many previous studies reported the isolation of rhizobia from *C. cajan* root nodules (Dubey *et al*., 2010; Shende & Patil, 2011). Although many previous works reported the characterization of rhizobia associated with cultivated legumes, the characterization of rhizobia associated with wild legumes is relatively very less. Wild legumes have potential for nitrogen fixation, reforestation and to control soil erosion (Lorite *et al*., 2010; Zahran, 2001). In addition wild legumes and their symbiotic partners have tolerance to extreme environmental conditions such as severe drought, salinity and elevated temperatures. Considering the above mentioned factors, in the present study the wild legumes *C. pallida* and *M. pudica* were investigated for determining the presence of beneficial rhizobia in their root nodules. *C. pallida* plants are annual shrubs, useful in agriculture as fodder crop and as a green manure (Magingo, 1992). Literature review suggested that work related to the characterization of rhizobia associated with *C. pallida* plant is very limited (Gao *et al*., 1994). In particular no documented work has been reported regarding the characterization of rhizobia from root nodules of *C. pallida* growing in Assam. Nodulation in the genus *Mimosa* is common and very widely reported (Chen *et al*., 2001; Barrett & Parker, 2006; Liu *et al*., 2007). It has long been known that *M. pudica,* is nodulated by diverse rhizobia and that it fixes large quantities of nitrogen (Mishra *et al*., 2012; Liu *et al*., 2012). Many previous studies suggested that rhizobia belonging to the subclass beta-proteobacteria are the major symbiont associated with *M. pudica* (Moulin *et al*., 2001; Gyaneshwar *et al*., 2011; Chen *et al*., 2003).

5.1. Isolation of rhizobia, phenotypic characterization and confirmatory tests

In the present study root nodulewas collected from 8 different geographical sites of Assam (Cachar, Karimganj, Hailakandi, N.C. Hills, Sivasagar, Tezpur, Kamrup and Kokrajhar) and used for the isolation of rhizobia. The sample collection spots were chosen on the basis of geographical location and local agronomic practices.

The attempts yielded 120 bacterial isolates.The primary cultures of bacteria obtained from the selected plants were investigated for their phenotypic features. Many previous researchers' emphasized on the classical phenotypic characterization of rhizobia which is helpful for primary screening of rhizobia (Sadowsky *et al*., 1983; Sharma *et al*., 2010). In the current study all the isolates formed white or off white color, round shaped slimy colonies on YEMA plates. The colony size of most of the isolates ranges from 1 to 3 mm in diameter after 3–5 days of incubation at 28^0C, however isolates obtained from *M. pudica* plants showed smaller size colonies (0.5 – 2 mm). The isolates also produced a significant amount of mucous which might protect them to withstand adverse environmental effects. The morphological features showed by the isolates resembles the standard morphological features of rhizobia (Holt *et al*., 1994) and the reference strains investigated in the present study also showed similar features. In addition, many previous researchers isolated rhizobia from root nodules of leguminous plants which showed similar morphological features.

Costa *et al*. (2014) reported that bacterial colonies isolated from *C. cajan* root nodules showed white color colonies with a maximum colony size of 3 mm (Costa *et al*., 2014). Similarly, Saha & Haquue, (2005) isolated bacterial colonies from *L. purpureus* root nodules, which were circular in shape with white color colonies on YEMA medium (Saha & Haque, 2005).

The isolates were investigated for their microscopic features by performing gram staining. Usually rhizobia shows gram negative staining and rod shape cells (Vincent, 1970). In the current study among 120 isolates investigated, 100 isolates (83%) showed gram negative staining and 20 isolates (17%) showed gram positive staining. The gram negative isolates include, 27 isolates obtained from *L. purpureus*, 27 isolates obtained from *C. cajan*, 23 isolates obtained from *C. pallida* and 23 isolates obtained from *M. pudica*. Among 120 isolates investigated, 108 isolates (90%) showed rod shape cells and 12 isolates (10%) showed coccus shaped cells. The isolates which showed rod shape cell includes, 28 isolates obtained from *L. purpureus*, 29 isolates obtained from *C. cajan*, 27 isolates obtained from *C. pallida* and 24 isolates obtained from *M. pudica*. The microscopic features of most of the isolates coincide with the standard features of rhizobia and with the microscopic features of reference strains (Vincent, 1970). Many recent studies also reported the isolation of rhizobia with similar microscopic features (Edulamudi *et al*., 2015; Bhargava *et al*., 2016).

For confirming the identity of the isolates as rhizobia 2 basic confirmatory tests (growth on YEMA-Cr medium and ketolactose test) were performed. Previous reports suggested that rhizobia usually appear as white color colonies on YEMA-Cr media (Sharma *et al*., 2010). In agreement with the previous studies, the present study revealed that most of the isolates do not absorb the congo red dye when grown on

YEMA-Cr media and appeared as white color colonies. However few isolates or contaminants absorbed the dye and appeared as red color colonies. Among the 120 isolates investigated, 104 isolates (89%) showed white color colonies and 16 isolates (11%) showed red color colonies on YEMA-Cr media. Similar to the present study, Amel *et al*. (2015) reported that rhizobia isolated from root nodules of *L. purpureus* plant did not absorb congo red dye (Amel *et al*., 2013).

Ketolactose test is usually performed for differentiating rhizobia from *Agrobacterium*. Generally the isolates which shows positive ketolactose test were considered as *Agrobacterium* and the isolates which shows negative ketolactose test were considered as rhizobia (Sharma *et al*., 2010). In the present study most of the isolates and reference strains were found to be negative for the production of 3-ketolactose from lactose. In these isolates after the addition of the Benedict's reagent, no halo zone was observed around the spot of inoculation. However, few isolate showed formation of halo zone. Among 120 isolates investigated, 101 isolates (84%) showed negative ketolactose test and 19 isolates (16%) showed positive ketolactose test. In recent past, Ansari *et al*. (2014) isolated 30 rhizobial isolates which showed negative results of ketolactose test from root nodules of soybean plant cultivated in India(Ansari *et al*., 2014). The isolates which showed absorption of congo red dye and positive ketolactose test were excluded from the study as the results were in contradiction to the standard features of rhizobia. Previous studies reported that the isolates which absorbed congo red dye mostly belongs to free living nitrogen fixers genera such as *Bacillus*, *Pseudomonas*, etc. and the isolates which show positive results of ketolactose test usually belong to *Agrobacterium* genera. Thus, based on the results of primary screening 28 isolates were excluded and 92 probable rhizobial isolates were selected for further study.

5.2. Biochemical characteristics

The ability to produce different enzymes to utilize various organic substrates is an important biochemical feature of rhizobia. The variation in enzymatic activities of rhizobia was reported by many previous researchers' (Bhargava *et al*., 2016; Kumari *et al*., 2010). The present study also revealed wide variation in the results of biochemical tests (Catalase, Oxidase, Nitrate, Starch, Urease, Citrate and Gelatinase test).

Catalase enzyme plays a protective role by splitting the toxic hydrogen peroxide into water and oxygen. It was also reported catalase activity plays an important role in nitrogen fixation metabolism. The result of the current study revealed that all the selected 92 (100%) isolates showed positive results of catalase test, which indicated that the isolates were capable of producing catalase enzymes. Upon addition of hydrogen peroxide on the slides of isolates oxygen bubbles were evolved, indicating the production of the catalase enzyme. Similar to the present study, many previous researcher's reported positive catalase activity of rhizobia isolated from different legume plants (Kumari *et al*., 2010; Patil *et al*., 2014). Oxidase positive bacteria possess cytochrome oxidase enzyme which plays an important role in the transfer of electrons from electron donors such as NADH to electron acceptor such as oxygen. Similar to the results of catalase test, all the selected 92 isolates (100%) showed positive results of oxidase test. In oxidase test smearing of isolates on oxidase disc results in blue coloration of the discs which indicated the production of oxidase enzyme. In agreement with the present study many previous reports supported the positive oxidase activity of rhizobia (Dubey *et al*., 2010).

The nitrate reduction test determines the production of nitrate reductase enzyme which reduces nitrate to nitrite. The nitrate reductase activity also plays an important role in nitrogen fixation metabolism. In nitrate test among the 92 isolates, 60 isolates (65%) showed positive results and 32 isolates (35%) showed negative results. The isolates which showed positive results of nitrate reduction test changes the color of the nitrate broth to blue after the addition of nitrate reagent. Starch hydrolysis test confirms the ability of bacteria to utilize starch as a carbon and energy source. The present study also revealed that all the 92 (100%) isolates showed negative results for starch hydrolysis test. In starch hydrolysis test no halo zone was formed around colonies of isolates upon addition of iodine reagent, indicating inability of the isolates The negative results of starch hydrolysis by rhizobial isolates are fully supported by many previous studies (Kumar *et al*., 2012).

Urease test determines the ability of isolates to produce urease enzyme which splits the urea present in media to ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate, which turns the medium from its original orange yellow color to bright pink. Previous studies also reported that urease enzyme plays an important role in nodule formation. In urease test 76 isolates (82%) showed positive results and 16 isolates (18%) showed negative results. Similar to the present findings, many previous studies reported the production of urease enzyme by rhizobia (Sadowsky *et al*., 1983; Deshwal & Chaubey, 2014). Citrate test confirms the ability of the isolates to utilize citrate as a source of carbon and energy. The result of the current study confirms that, most of the isolates couldn't utilize citrate as a carbon source, except few isolates obtained from *M. pudica*. Among the 92 isolates investigated, 35 isolates (38%) showed positive results for citrate utilization and 55 isolates (62%) showed negative results. The isolate which utilizes citrate as a carbon source showed visible growth in citrate media and changes the color of the media to deep blue.

The gelatin hydrolysis test is used to detect the ability of microorganisms to produce the enzyme gelatinase. Gelatinase enzyme plays an important role in nodule formation. In gelatin hydrolysis test 21 isolates showed positive result (22%) and 71 isolates (78%) showed negative result. It was observed that gelatianse test negative isolates do not produce gelatinase enzymes as medium containing gelatin solidified when kept at 4°C. Negative gelatinase activity is also a common feature of rhizobia (Singh *et al*., 2006). The isolates which showed positive result secrete gelatinase which liquefy the gelatin, resulting in the liquefaction of the medium. Similar to the result of present study, Hunter *et al*. (2007) reported negative gelatinase activity by *Rhizobium* isolates (Hunter *et al*., 2007).

5.3. Plant growth promoting abilities

Rhizobia are well known as a plant growth promoter. Many indigenous rhizobial strains with the plant growth promoting ability have already been identified and characterized from different legume plants. Datta and Basu, (2000) isolated plant growth promoting rhizobialstrains from root nodules of *S. procumbens, S. rostrata, C. cajan* (Datta & Basu, 2000). Pandey *et al*. (2005) isolated plant growth promoting root nodule harboring rhizobialstrains from *M. pudica* (Pandey *et al*., 2005).In the present study, plant growth promoting ability of the isolates was determined by 3 different tests viz., IAA production, phosphate solubilization and ammonia production.

IAA is an important plant hormone and plays a significant role in plant growth. Production of IAA by rhizobiaisolated from legume plantshas been previously reported by many researcher's (Dubey *et al*., 2010). It is well documented in earlier studies that the amount of IAA production varies with species, strains, growth conditions andlocation. The present study revealed that among 92 isolates investigated, 72 (78%) isolates produced IAA and the amount of IAA produced varied from 13 µg/ml to 77 µg/ml. The isolates which produced IAA includes, 17 isolates obtained from *C. cajan*, 20 isolates obtained from *L. purpureus*, 17 isolates obtained from *C. pallida* and 19 isolate obtained from *M. pudica*. Based on the amount of IAA produced the isolates can be divided into two groups low IAA producers (10 – 40 µg/ml) and high IAA producers (41 – 80 µg/ml). It was found that 43% of isolates belong to a low IAA producer group and 57% of isolates belong to a high IAA producer group. Among 72 isolates that produced IAA, 5 *L. purpureus* isolates (RDL1, KDL1, HIDL1, DDL2 and SDL1), 3 *C. cajan* isolates (SICC1, SICC3 and TECC3), 6 *C. pallida* isolates (MKCP1, MKCP2, SICP2, DCP1, SCP1 and HCP2) and 4 *M. pudica* isolates (HMP1, BMP1, SIMP1 and SMP2) produced higher amount of IAA than reference strain *R. leguminosarum*. The highest amount of IAA production was showed by SDL1 (77 µg/ml) isolated from *L. purpureus* grown in Tezpur, followed by HMP1 (73 µg/ml) isolated from *M. pudica* grown in Cachar and MKCP1 (66 µg/ml) isolated from *C. pallida* grown in Karimganj. Many previous works reported the production of IAA by rhizobial isolates from different legume plants (Sridevi *et al*., 2007). The present study was also complementary with the results of Dubey *et al*. (2010), who reported the characterization of IAA producing rhizobial isolates from root nodules of *C. cajan* (Dubey *et al*., 2010).

Phosphorus is anecessary plant nutrient. Inorganic phosphorus present in the soil is unavailable to the plants due to its inert nature. This inorganic phosphorus is solubilized by many, microorganisms including rhizobia (Dubey *et al*., 2010, Bhargava *et al*., 2016). During phosphate solubilization microorganisms releases organic acids, which will reduce the media pH and consequently solubilize the insoluble tricalcium phosphate. Results of the present study confirmed that most of the isolates were capable of performing phosphate solubilization. Phosphate solubilization is confirmed by the formation of the solubilization zone around the spot of inoculation of isolates on Pikovskaya's agar plates. PSE of the isolates was determined by comparing the colony diameter of the isolates with the diameter of the solubilization zone. The present study revealed that among 92 isolates investigated, 75 isolates (81%) sloubilizes phosphate. The isolates which solubilizes phosphate includes, 20 isolates obtained from *L. purpureus,* 18 isolates obtained from *C. cajan,* 18 isolates obtained from *C. pallida* and 19 isolates obtained from *M. pudica*. Based on the ability of phosphate solubilization the isolates can be grouped into two groups low phosphate solubilizers (100 – 150 PSE) and high phosphate solubilizers (150 – 200 PSE). Among the 75 isolates, 3 isolates of *L. purpureus* (KDL1, IDL1 and DDL2), 3 isolates of *C. cajan* (ICC1, SICC3 and SICC3), 7 isolates of *C. pallida* (RCP2, KACP2, SICP2, DCP1, NCP1, NCP2 and SCP1) and 8 isolates of *M. pudica* (IMP1, HMP1, BMP1, GMP1, NCMP1, DMP2, SMP2 and KOMP2) showed higher phosphate solubilization efficiency than reference strains. The maximum PSE was showed by DCP1 isolated from *C. pallida* grown in N.C. Hills, followed by IDL1 isolated from *L. purpureus* grown in Cachar and RCP2 isolated from *C. pallida* grown in Cachar. Similar to the present results, Dubey *et al*. (2010) isolated *Sinorhizobium* strains from root nodules of *C. cajan* which can solubilize inorganic phosphate (Dubey *et al*., 2010). Very recently, Bhargava *et al*. (2016) reported the isolation of phosphate solubilizing rhizobia from *M. pudica* plant grown in semi-arid region of Tirupati, India (Bhargava *et al*., 2016).

Another important trait of PGPR is the production of ammonia that indirectly influences the plant growth. Many previous studies reported the production of ammonia by rhizobia isolated from legume plants (Kumar *et al*., 2012). The present study also confirmed that the majority of the isolates were capable of ammonia production. Among the 92 isolates, 71 (77%) isolates showed production of ammonia. These 71 isolates includes 17 isolates obtained from *L. purpureus*,18 isolates obtained from *C. cajan,* 18 isolates obtained from *C. pallida* and 18 isolates obtained from *M. pudica*. Development of faint yellow to dark brown color on peptone broth inoculated with the isolates indicated the production of ammonia. Similar to the present findings, Deb *et al*. (2015) isolated rhizobia from root nodules of *C. cajan* and reported that the isolates can successfully produce ammonia (Deb *et al*., 2015). Likewise, Bhargava *et al*. (2016) reported the production ammonia by root nodule harboring rhizobia isolated from different wild legumes including *M. pudica* (Bhargava *et al*., 2016).

5.4. Acidic pH and salt tolerance

Salinity and pH are major limiting factors restricting symbiotic nitrogen fixation. Isolation and characterization of indigenous rhizobial strains which can tolerate acidic pH and high salinity can play an important role in the selection of superior rhizobial strains. Slight variation in pH might have significant effects on the growth of bacteria. The fact that different strains of the same species may vary widely in their pH tolerance has been demonstrated previously (Glenn & Dilworth, 1994). In many previous findings it was reported that strains of *Rhizobium leguminosarum* are generally sensitive to low pH and grow on near neutral and basic pH (Graham *et al*., 1994; Jordan, 1984). Isolation of acidic pH tolerant rhizobia from different legume plant was previously reported by many researchers (Costa *et al*., 2014; Amel *et al*., 2013). Many studies from different countries like Kenya, Egypt, South Africa reported the isolation of rhizobia which can tolerate acidic pH in the range 3.0 – 5.0. (Zahran *et al*., 1999; Bordeleau *et al*., 1994).

It is also important to mention here that soil pH of the Northeastern region of India, particularly Assam is slightly acidic 4.2 - 5.8. Many previous reports suggested the presence of acidic pH tolerant rhizobia from root nodules of legume plants cultivated in Assam (Choudhury *et al*., 2010). Selection of acid tolerant rhizobia to inoculate legume hosts under acidic conditions may help the establishment of the symbiosis and also may improve the acid tolerance of legume. In a previous work, Costa *et al*. (2014) reported that rhizobia isolated from *C. cajan* grown in the eastern edge of Brazilian pantanal can survive at pH 4.0 (Costa *et al*., 2014). Likewise, Amel *et al*. (2013) reported tolerance of a few strains of rhizobia associated with *L. purpureus* to pH 4.0 (Amel *et al*., 2013). The results of the present study clearly indicated that the isolates grew well on YEMA media adjusted at pH 5.0 and 6.0, but unable to survive at pH 4.0. Among the 92 isolates investigated all the isolates (100%) showed visible growth at pH 6.0 and 50 isolates (41%) showed growth at pH 5.0. The present finding corroborated with the studies of many earlier workers who reported that the optimum pH for the growth of rhizobia lies at pH 6.0 – 7.0 (Singh *et al*., 2008; Gauri *et al*., 2011). It was also found that 5 isolates survived and showed growth at pH 4.0. These 5 isolates were obtained from the root nodules of *C. pallida* (KACP2 andGCP1) and *M. pudica* (RMP2, SIMP3 and KMP2). In contrast to the previous studies the isolate obtained from the root nodules of *L. purpureus* and *C. cajan* showed no growth at pH 4.0. Isolation of rhizobial strains which can survive at pH 4.0 and pH 5.0 indicated that the isolates can be used as an effective inoculant in acidic soil. Isolation of rhizobia with a wide range of pH tolerance is a pavement to acquire effective native rhizobia tolerant to acidic soil pH.

Salt stress or salinity significantly reduces nitrogen fixation and nodulation in legumes. Hashem *et al*. (1998) proposed that, salt stress may decrease the efficiency of the symbiosis by reducing plant growth and photosynthesis (Hashem *et al*., 1998). Salt tolerant rhizobia may have the potential to improve yield of legumes under salinity stress. The adaptation to high salinity could be attributed to the accumulation of low molecular weight organic solutes called osmolytes that prevent the cell lysis (Csonka & Hanson,1991). The results of the present study revealed that, most of the isolates survive and showed visible growth on YEMA media containing 1% and 2% NaCl (w/v). However, at higher concentrations the numbers of tolerant strains decreased rapidly and only a few strainssurvivedon YEMA media containing 3% NaCl. Among the 92 isolates investigated, all the isolates (100%) showed visible growth on YEMA media containing 1% NaCl and 59 isolates (49%) showed growth on YEMA media containing 2% NaCl. However, at 3% NaCl only 4bacterial isolates (RCP2, KACP2, SICP2 and SMP2) survived and showed growth. RCP2, KACP2 and SMP2 were isolated from root nodules of *C. pallida* collected from Cachar and Hailkandi. SMP2 was isolated from root nodules *M. pudica* collected from Tezpur. Decrease in growth of rhizobial isolates with increased salt concentration was also reported in many previous studies (Rai *et al*., 2012; Pandey *et al*., 2005). This decrease is considered to be due to toxicity and also due to osmotic stress imposed by salinity. Similar to the present finding, Pandey *et al*. (2005) reported that *Burkholderia* sp. MSSP a root nodulating bacteria isolated from *M. pudica* survive on 1% and 2% NaCl (Pandey *et al*., 2005). Likewise, Fernandes Júnior *et al*. (2012) reported the isolation of rhizobia from root nodule of *C. cajan* which can tolerate 1-3% NaCl (Fernandes Júnior *et al*., 2012). The results of the present study also coincide with the findings of Rasul *et al*. (2012) who reported isolation of rhizobial isolates which tolerate 3% NaCl (Rasul *et al*., 2012). The results clearly indicated that the isolates which tolerated high NaCl concentration (2 - 3% NaCl) could be used as effective inoculants in agricultural soils effected with high salinity.

5.5. *nifH* and *nodC* gene amplification

Symbiotic (*nif* and *nod*) genes are located in easily interchangeable elements (plasmids or in symbiotic islands) and required for the successful establishment of symbiosis between rhizobia and legumes. Rhizobia are well known nitrogen fixers due to the presence of *nif* genes which encodes the nitrogenase enzyme (Chang *et al*. 2011). Nitrogenase is the main enzyme associated with biological nitrogen fixation. In addition to rhizobia *nif* genes are found in many free living nitrogen fixing bacteria. By the amplification of some specific *nif* genes such as *nifH,* the nitrogenase activity of the rhizobia can be confirmed (Young& Haukka, 1996; Pandey *et al*., 2004). In the present study 40 selected isolates were investigated for the presence of *nifH* genes in their genomes. PCR amplification reaction using *nifH* primers generated specific bands at 360 bp confirming the presence of *nifH* gene. Among 40 isolates, 29 isolates showed amplification of *nifH* gene. It includes, 7 isolates obtained from *L. purpureus*,6 isolates obtained from *C. cajan*, 6 isolates obtained from *C. pallida* and10 isolates obtained from *M. pudica*. In the PCR amplification reaction reference strain *R. leguminosarum* MTCC-99 was used as a positive control. Similar to the present findings, many previous works reported the presence of *nifH* in the rhizobial isolates of *M. pudica*, *C. cajan*, *L. purpureus* (Klonowska *et al*., 2012;Dubey *et al*., 2010; Chang *et al*., 2011). The present study also revealed the presence of *nifH* gene in some species of *Burkholderia*, which belong to beta-subclass ofproteobacteria. It was believe that rhizobia that belong to beta–proteobacteria group had acquired the *nif* gene by horizontal transfer from their counterpart alpha-proteobacteria (Hurek *et al*., 1997). The presence of *nif* genes in rhizobia of beta-proteobacteria group *viz.*

Burkhiolderia, *Ralstonia*, was reported in many previous works (Verma *et al*., 2004). The absence of *nifH genes* in some isolates may be explained in several ways, including the presence of unique *nif* elements that required to be characterized by using different primers. However, there is also the possibility that these isolates may lack nitrogen fixing ability and possibly are only opportunistic endophytic in nature.

Bacterial genes that function in nodulation are termed nodulation or *nod* genes and their expression is induced by flavonoid signals from the host plant. To form an effective symbiosis, rhizobia require several classes of nodulation genes. These include *nod, nol, noc* genes which encode the production of nod factors and which stimulate the plants to produce symbiotic nodules. *nodC* gene encodes N-acetylglucosaminyltransferase enzyme that synthesizes the N-acetyl glucosamine backbone of the nod factor. The *nod*ABC genes are responsible for the synthesis of the chitin backbone. The common *nod*ABC genes and the regulatory *nod*D1 and *nod*D2 genes of rhizobia have already been characterized in many previous studies (Appelbaum *et al*., 1988). Amplification of nod genes has also been reported by earlier workers and proved their role in nodule formation by several species of rhizobia (Haukka *et al*., 1998; Kumar *et al*., 2006). The results of the current study revealed the presence of *nodC* gene in 27 isolates. These include 7 isolates obtained from *L. purpureus*, 6 isolates obtained from *C. cajan*, 4 isolates obtained from *C. pallida* and 10 isolates obtained from *M. pudica*. PCR amplification reaction using *nodC* primers generated specific bands at 500 bp, confirming the presence of *nodC* gene. In the amplification reaction reference strain *R. leguminosarum* MTCC-99 was used as a positive control. Presence of *nodC* gene in rhizobial isolates of *C. cajan* and *L. purpureus* was previously reported by many researcher's (Dubey *et al*., 2010; Chang *et al*., 2011). Interestingly, the beta-proteobateria isolates obtained in the present study also showed the presence of *nodC* gene. In accordance with the present result, it has been proposed earlier that *Mimosa* spp. nodulating *Burkholderia* strains, obtained their *nod* genes via multiple lateral gene transfer from alpha-rhizobia (Moulin *et al*., 2001;Vandamme *et al*., 2002). It is also important to note that few isolates did not showed amplification of *nod* gene, even though they have showed all the standard features related to rhizobia. The possible reason for this might be that the primers used were not suitable for the strains or these strains may have some other mechanism to induce nodule formation. Alternatively the isolates might be an endophytic bacteria in the root nodules. In fact in the present study two isolates DCP1 and NCP1, which were identified as endophytic *Bacillus* strains did not showed amplification of *nodC* gene.

5.6. 16S rDNA PCR-RFLP analysis

16S rDNA PCR-RFLP analysis is a useful technique for studying genetic diversity of rhizobia. The sequence of the 16S rDNA had been widely used as a phylogenetic marker to study genetic relationships between different species of bacteria. The analysis of this gene can therefore be considered as a standard method for the identification of bacteria in the family, genus and even sometimes at species levels (Weisburg *et al*., 1991). 16S rDNA PCR-RFLP analysis is a reliable, rapid and practical method used for phylogenetic relationships among and within closely related species. Many previous researchers reported the characterization of rhizobial strains based on 16S rDNA PCR-RFLP analysis (Verma *et al*., 2004; Chang *et al*., 2011; Costa *et al*., 2014). Edulamudi *et al*. (2015) characterized rhizobial strains isolated from Horse gram from different regions in Andhra Pradesh and Telangana states, India based on 16S rDNA PCR-RFLP analysis (Edulamudi *et al*., 2015). In the present study, 16S rDNA region of the 40 selected isolates and reference strains were

PCR amplified with universal primers 27F and 1492r. The PCR reaction generated specific bands at 1500 bp which confirmed the amplification of 16S rDNA. The PCR products were individually restricted with different restriction enzymes. The results also demonstrated a considerable degree of genetic diversity among the isolates. A number of well separated,sharp bands ranging from 200 - 1000 bp were detected, clearly showing the differences between the isolates and the reference strains.

On the basis of restriction pattern of 16S rDNA an UPGMA dendrogram is generated. Dendrogram derived from the PCR-RFLP analysis of isolatesobtained from *L. purpureus* generated 2 different clusters. Cluster I which comprised of 7 isolates formed the predominant group and showed close resemblance with 2 reference strains *R. leguminosarum* MTCC 99 and *B. japonicum* MTCC 120. On the other hand cluster II comprised of 3 isolates and showed closest similarity with reference strain *M. thiogangeticum* MTCC 7001. Similar to the present study, Chang *et al*. (2010) employed PCR-RFLP technique for characterizing rhizobial isolates obtained from *L. purpureus* (Chang *et al*., 2010). PCR-RFLP analysis of isolatesobtained from *C. cajan* revealed a considerable diversity among the PCR-RFLP patterns. Based on the dendrogram derived from PCR-RFLP, the isolates were grouped into2 different clusters. In this case 4 isolates clustered with *B. japonicum* and forms cluster I, the remaining 6 isolates group with *R. leguminosarum* and *M. thiogangeticum* and forms cluster II. In recent past, Costa *et al*. (2014) also characterized rhizobia from *C. cajan* based on PCR-RFLP analysis by employing 3 restriction enzymes *Dde*I, *Msp*I, and *Hinf*I (Costa *et al*., 2014). The results of PCR-RFLP analysis revealed that *C. pallida* isolates can be divided into 3 different clusters. Cluster I comprised of 3 isolates and showed closest association with *B. japonicum*, cluster II comprised of 6 isolates and showed closest association with *R.*

leguminosarum, cluster III comprised of 2 isolates and showed closest association with *M. thiogangeticum*. For restriction digestion of 16S rDNA of *M. pudica* rhizobial isolates *AluI* restriction enzyme was used. Dendrogram revealed the formation of 3 different clusters. Cluster I comprised of 4 isolates and showed a close association with *B. cepacia*, cluster II comprised of 5 isolates and cluster III comprised of 1 isolates and showed association with 3 reference strains *M. thiogangeticum*, *R. leguminosarum* and *B. japonicum* respectively. In a previous study,Verma *et al*. (2004) reported that *AluI* is the most significant restriction enzyme for digesting *M. pudica* isolates (Verma *et al*., 2004).

5.7. 16S rDNA sequencing analysis

Although phenotypic methods play a significant role in identification, molecular tools are more reliable and authentic for identification and to study the genetic diversity of bacterial isolates. Currently, it is known that 16S rDNA gene analysis is the most suitable method used to classify new strains of rhizobia. Comparison of 16S rDNA gene sequences are useful forassigning species to genera and for defining inter and intra-generic relationships. 16S rDNA sequence analysis has been used for bacterial identification at the genus and species levels as well as for inferring phylogenetic relationships among prokaryotic organisms. As a general rule, bacterial strains that exhibit 16S rDNA gene sequence similarities <97 % relate to different species, while those with sequence similarities >97 % most likely, but not necessarily, belong to the same species (Stackebrandt & Goebel, 1994).

For genotypic characterization, 4 rhizobia isolates obtained from *L. purpureus* (HIDL1, SDL1, SIDL1 and DDL2) which represents 2 different PCR-RFLP clusters were selected for 16S rDNA gene sequencing. Sequencing results revealed that irrespective of different PCR-RFLP patterns, all the isolates obtained from *L.*

purpureus showed close similarity with the strains of *Bradyrhizobium* genus in BLAST analysis. The genus *Bradyrhizobium* mostly represents the slow growing rhizobia and strains belonging to this genus were isolated from a broad range of legume plants. BLAST analysis revealed that HIDL1 and DDL2, isolated from Hailakandi and N.C. Hills regions showed 99% sequence similarities with *Bradyrhizobium elkanii* NBRC 14791. *B. elkanii*, a common legume symbiont, originally diverged from *B. japonicum* and previously isolated from root nodules of many legume plants (Kuykendall *et al*., 1992). Results of the present study also coincides with the results Chang *et al*. (2011) who reported the isolation *B. elkanii* from root nodules of *L. purpureus* (Chang *et al*., 2011). SDL1, isolated from Tezpur showed 99% sequence similarity with *Bradyrhizobium lablabi* CCBAU 23086.*B. lablabi* is also a common legume symbiont and was first reported from root nodules of *L. purpureus* (Chang *et al*., 2011). It was also reported that phenotypic and genotypic features of *B. lablabi* were most closely related to *B. jicamae*, *B. pachyrhizi* and *B. elkanii*. SIDL1 isolated from Hailakandi showed 98% sequence similarity with *Bradyrhizobium pachyrhizi* PAC48. *B. pachyrhizi* was first isolated from root nodules of *Pachyrhizus erosus* in 2009, and in later years they were isolated from many other legume plants (Ramírez-Bahena*et al*., 2009).The result of the current study is in agreement with the findings of Chang *et al*. (2011) who reported the presence of *B. elkanii*, *B. lablabi*, *B. pachyrhizi*, *B. yuanmingense*, *B. jicamae* as a symbiont of *L. purpureus* (Chang *et al*., 2011). They also reported that among the *Bradyrhizobium* spp. associated with *L. purpureus*, *B. elkani* was the most dominant species. Although the present study indicated that rhizobia from *Bradyrhizobium* genus are the most common symbiont associated with *L. purpureus* plant, presence of rhizobia from *Sinorhizobium* and *Mesorhizobium* genera in the root nodules of *L. purpureus* has also been reported in few previous studies. In 1980, Trinick reported the presence of fast growing *Sinorhizobium fredii* along with other slow growing rhizobia from root nodules of *L. purpureus* grown in Papua New Guinea (Trinick, 1980). Likewise, Amel *et al*. (2013) reported the presence of *Bradyrhizobium* and *Mesorhizobium* strains in the root nodules of *L. purpureus* isolated from 3 different locations of Algeria (Amel *et al*., 2013). The acquired 16S rDNA sequences of the 4 isolates were deposited in GenBank database and the strains were named as *Bradyrhizobium elkanii* HIDL1, *Bradyrhizobium lablabi* SDL1, *Bradyrhizobium pachyrhizi* SIDL1 and *Bradyrhizobium elkanii* DDL2. To confirm the phylogenetic relationship of the isolates, a neighbor joining phylogenetic tree was constructed on the basis of 16S rDNA partial gene sequences of the 4 isolates and reference sequences obtained from GenBank by ClustalW alignment method. The phylogenetic tree revealed the formation of 3 different clusters. The phylogenetic tree analysis coincides with the results of BLAST analysis, the isolates HIDL1, SDL1, SIDL1 and DDL2 forms a single cluster with *B. elkanii*, *B. lablabi*, *B. pachyrhizi*.

Many previous reports suggested the presence of fast and slow growing rhizobia in the root nodules of *C. cajan.* Ramsubhag *et al*. (2002) reported the isolation of slow growing *Bradyrhizobium elkanii* from root nodules of *C. cajan* (Ramsubhag *et al*., 2002). Likewise, Dubey *et al*. (2010) reported the presence of plant growth promoting strains of *Sinorhizobium freedi* from root nodules of *C. cajan* (Dubey *et al*., 2010). Recently, Costa *et al*. (2014) isolated and characterized fast growing rhizobial strains from *C. cajan* plant growing in Brazil (Costa *et al*. 2014). In the present study 16S rDNA sequencing analysis revealed that *C. cajan* symbionts belongs to 3 different genera *Rhizobium*, *Mesorhizobium* and *Burkholderia*. In BLAST analysis the isolate DCC2 from N. C. Hill showed 98% sequence similaritywith *Rhizobium mayense*. Previously, *R. mayense* was reported to be isolated from *Calliandra grandiflora* growing in Mexico (Rincon-Rosales *et al*., 2013). BLAST results revealed that ICC1 from Cachar showed 99% sequence similaritywith *Mesorhizobium loti*. Many previous reports suggest the presence of *M. loti* as a symbiont of leguminous plants. Kang *et al*. (2012) reported the characterization of *M. loti* isolates from leguminous trees growing in South Korea (Kang *et al*., 2013). Likewise, Rai *et al*. (2012) reported the presence of *M. loti* in the root nodule of *Cicer arientum* L. grown in India (Rai *et al*., 2012).

The present study also revealed that 2 isolates, ICC3 and SICC1 isolated from Cachar and Hailakandi belongs to *Burkholderia* genus. The genus *Burkholderia* is one of the largest groups of species within the class beta-proteobacteria and currently comprises more than 60 species. They are distributed in diverse habitats such as plant rhizosphere, in plant root as endophytes, in root nodules as symbiont or can be present as opportunistic pathogens on plants and humans. Many species of *Burkholderia* are capable of fixing atmospheric nitrogen and can also act as a potent plant growth promoter (Chen *et al*., 2005). Pandey *et al*. (2005) reported the isolation of plant growth promoting *Burkholderia sp.* MSSP from root nodule of *Mimosa pudica* (Pandey *et al*., 2005). In the present study, ICC3 from Cachar and SICC1 from Hailakandi showed 99% sequence similarity with *Burkholderia cenocepacia* and *Burkholderia anthina* respectively. *B. cenocepacia* and *B. anthina* belongs to *Burkholderia cepacia* complex. *B. cepacia* complex consists of 17 different species and are responsible for potentially lethal pulmonary infections in patients with cystic fibrosis (Mahenthiralingam *et al*., 2000). Although strains of *B. cepacia* complex was mostly considered as a human pathogen, but they also include free-living soil bacteria and nitrogen fixing symbiotic bacteria (Miller *et al*., 2002). Rasolomampianina *et al*.

(2005) isolated and characterized strains of *B. cepcia* from root nodule of *Dalbergia louveli* growing in central-southern regions of Madagascar (Rasolomampianina *et al*., 2005). Likewise, Singh *et al*. (2006) reported the presence of endophytic *B. cepacia* strains from root of *Oryza sativa* and also confirm the presence of *nif* and *nod* genes in this isolates (Singh *et al*., 2006). In recent past, Lu *et al*. (2012) isolated strains of *B. stabilis* and *B. cepacia* both of which are included in *B. cepacia* complex from root nodules of *Dalbergia odorifera* (Lu *et al*., 2012). The presence of *B. cenocepacia* and *B. anthina* in the nodules of *C. cajan* may likely to be considered as an opportunistic interaction by the microorganisms. The acquired 16S rDNA sequences of the 4 isolates were deposited in GenBank database and the strain were named as *Mesorhizobium loti* ICC1, *Burkholderia cenocepacia* ICC3, *Rhizobium mayense* DCC2 and *Burkholderia anthina* SICC1.For determining the evolutionary relationship a phylogenetic tree was constructed from 16S rDNA sequence of *C. cajan* isolates and reference sequences by using the ClustalX program.

In earlier studies it was found that rhizobial strains associated with *Crotalaria* mostly belong to the genus *Bradyrhizobium*. Samba *et al*. (1999) reported the presence of slow growing *Bradyrhizobium* strains from *Crotalaria* spp. collected from different geographical sites of Senegal (Samba *et al*., 1999). Liu *et al*. (2007) isolated and characterized rhizobia from *Crotalaria pallida* grown in China and reported the presence of 4 different genera *Rhizobium*, *Bradyrhizobium*, *Burkholderia* and *Methylobacterium* (Liu *et al*., 2007). However, in contrast to the previous studies, results of the present study revealed that rhizobia associated with *C. pallida* belong to *Mesorhizobium* and *Bacillus* genera. NCBI-BLAST analysis confirms that among the 4 isolates identified 2 isolates RCP2 and KACP2 from Cachar, showed 99% sequence similarities with *Mesorhizobium jarvisii* and 97% sequencesimilarities with

Mesorhizobium jarvisii. Species belonging to *Mesorhizobium* genus are common legume symbionts and isolated from many legume plants from different regions of the world. The species *M. jarvisii* is converted from *M. loti* ATCC 33669T which was originally isolated from *Lotus corniculatus*. Martinez-Hidalgo *et al*. (2015) reported that the genotypic features of a few strains of *Mesorhizobium viz. M. loti* ATCC 33669T and *M. loti* USDA 3471T do not coincide with their original type strain *M. loti* NZP 2213T (Martinez-Hidalgo *et al*., 2015). The analysis of the 16S rRNA gene showed that strains USDA 3471T and ATCC 33669T formed independent branches from *M. loti* NZP 2213T. Thus, these 2 strains were reclassified as representatives of the two species *M. jarvisii* sp. nov. and *M. erdmanii* sp. nov. DCP1 and NCP1 isolated from N.C. Hills, showed 97% sequence similarities with *Bacillus megaterium* and 97% sequence similarities *Bacillus koreensis* respectively. Many previous studies reported that apart from rhizobia other non-rhizobial endophytic bacteria may infect the root nodules of legume plants (Dudeja *et al*., 2012; Saini *et al*., 2015; Kumar *et al*., 2013). The term microbial endophyte is applied to the microorganisms that are isolated from plant parts viz., root, stem, leaf after surface sterilization. Several reports have shown that endophytic bacteria exhibit overall plant growth enhancement via different mechanisms (Rashid *et al*., 2012; Ji *et al*., 2014). Similar to the present findings Khalifa and Almalki, isolated and characterized endophytic bacterium *Bacillus megaterium* from surface sterilized root nodules of *Medicago sativa* (Khalifa & Almalki, 2014). The prevalence of non-nodulating isolates in nodule extracts may be explained by different alternatives, including changes in the symbiotic genes due to mutation, opportunistic colonization by rhizospheric bacteria or surface contamination of nodules. However, since they survived after surface sterilization of nodules and represented the major phenotype recovered from nodule they will be considered as putative endophytes. The acquired 16S rDNA sequences of the 4 isolates were deposited in GenBank database and the strain were named as *Bacillus megaterium* DCP1, *Mesorhizobium jarvisii* RCP2, *Bacillus koreensis* NCP1 and *Mesorhizobium jarvisii* KACP2. For determining the evolutionary relationship a phylogenetic tree was constructed from 16S rDNA sequence of *C. Pallida* isolates and reference sequences by using the ClustalX program. The phylogenetic tree was in full agreement with the results of BLAST analysis. In the phylogenetic tree the isolates formed clusters with reference strains with which they showed a close similarity in BLAST analysis.

Many previous works on rhizobia associated with *M. pudica* reported that beta-rhizobia are the most common rhizobia present in the root nodules of *M. pudica* (Moulin *et al*., 2001; Gyaneshwar *et al*., 2011; Chen *et al*., 2003). Liu *et al*. (2011) reported that the beta-rhizobia belonging to the genera *Burkholderia* and *Cupriavidus* are the main nitrogenous bacteria associated with *M. pudica* in Sishuangbanna and Yuanjiang districts of Yunnan, China (Liu *et al*., 2011). Likewise, Klonowska *et al*. (2012) investigated the genetic diversity of rhizobia associated with *M. pudica* growing in New Caledonia and reported that majority of symbionts were *Cupriavidus taiwanensis* (Klonowska *et al*., 2012). The result of the present study fully supported the previous reports. In the present study, BLAST analysis of 16S rDNA sequences of *M. pudica* isolates clearly indicated that beta-rhizobia belonging to the genus *Burkholderia* were the most common symbionts of *M. pudica*. Among the 4 sequenced isolates, 3 isolates belongs to *Burkholderia* genus. SMP2, HMP1 and DMP2, isolated from Tezpur, Cachar and N.C. Hills showed close sequence similarities with *Burkholderia mimosarum* PAS44 with similarity level of 99%, 98% and 99% respectively. *B. mimosarum* was first reported by Chen *et al*. (2006) from the root nodules of *Mmosa pigra* and *Miomosa scabrella* from Taiwan, Venezuella and Brazil (Chen *et al*., 2006). However, in the present study no strains from *Cupravidus* genera had been characterized.The BLAST analysis also revealed that KMP1 isolated from Karimganj showed closest similarity with *Mesorhizobium loti* MAFF303099 which belongs to alpha-rhizobia with a similarity level of 98%. Although beta-rhizobia appear to be the predominant symbionts of *M. pudica*,the presence of alpha-rhizobia in close association with *M. pudica* had been previously reported by many researcher's (Barrett & Parker, 2006). In recent past, Klonowska *et al*. (2012) reported the presence of *Rhizobium mesoamericanum* from root nodules of *M. pudica* (Klonowska *et al*., 2012). The acquired 16S rDNA sequences of 4 isolates were deposited in GenBank database and the strain were named as *Burkholderia mimosarum* SMP2, *Burkholderia mimosarum* HMP1, *Burkholderia mimosarum* DMP2 and *Mesorhizobium loti* KMP1. Phylogenetic tree constructed by aligning the 16S rDNA sequences of *M. pudica* isolates and reference sequences from GenBank also supported the results of BLAST analysis. The phylogenetic tree coincides with the results of BLAST analysis. The isolates SMP2, DMP2 and HMP1 forms cluster with reference strains of *Burkholderia* genera, whereas the isolate KMP1 cluster with the strains of *Mesorhizobium* genera.

5.8. Nodulation study and assessment of plant growth

Rhizobia inoculation is a cheaper and usually a more effective agronomic practice for ensuring an adequate supply of nitrogen for legume based crop than the application of fertilizer nitrogen (Marufu *et al*., 1995). Inoculation of rhizobial strains has a significant effect on the metabolic activities of the plant. Many previous reports suggested that inoculation of leguminous and non-leguminous plants with rhizobia can significantly increase the growth and activity of plants (Lynch, 1990; Saxena *et* *al*., 2006; Maier & Triplett, 1999; Gupta *et al*., 1998). In the present study a pot experiment was carried out to evaluate the effect of isolates on their respective host plants. All the identified isolates along with the reference strain *R. leguminosarum* were inoculated on their host plants (*L. purpureus*, *C. cajan*, *C. pallida* and *M. pudica*) under sterilized condition. It was found that the ability of nodulation was in the range of 5-10 nodules/plant. The nodules were healthy and pink in color. It was also observed that the result of nodulation test is in agreement with the results of *nodC* gene PCR amplification. The isolates which showed amplification of *nodC* gene, successfully forms nodules on their host plants and the isolates which did not showed the amplification of *nodC* gene unable to form any nodules on their host plants. In the current study shoot length, root length, shoot dry weight and root dry weight were kept as a parameter for studying the effectiveness of isolate inoculation. Several reports showed that shoot dry mass and root dry mass is a good indicator of relative strain effectiveness and there is a good correlation between shoot dry matter production and nitrogen fixation capacity of legumes (Somasegaran & Hoben, 1994; Peoples & Craswell,1992).

Many previous studies reported the nodulation and plant growth promoting ability of rhizobia on *L. purpureus* (Saha & Haque, 2005; Amel *et al*., 2013). In the present study it was observed that inoculation of rhizobia isolates have generated significant effect on the vegetative parameters of *L. purpureus*. Inoculation of HIDL1 which was identified as *B. elkanii* significantly increase shoot dry weight (85.65 mg) of *L. purpureus* compared to un-inoculated control (59.00 mg). Likewise, inoculation of DDL2 which was also identified as *B.elkanii* significantly increases root length (7.00 mg) of *L. purpureus* when compared with un-inoculated control (4.89mg). Similar to the present study, Amel *et al*. (2013) reported the increase in root height and shoot dry weight after inoculation of rhizobial isolates on their host plant (Amel *et al*., 2013). Reference strain, *R. Leguminosarum* MTCC-99 significantly increases the root length and root dry weight of *L. purpureus*. In PGP test HIDL1 produced IAA, solubilized phosphate and in stress tolerance test it showed growth at pH 6.0 and 2% NaCl. Likewise, significant interaction between isolates and their host plants suggested that HIDL1 and DDL2 can be used as effective inoculants for their host plant. However, inoculation of SDL1 and SIDL1 has no significant effect on vegetative parameters of *L. purpureus*. Rhizobia isolated from *C. cajan* also induces root nodules on their host plant. The isolate DCC2 which was identified as *R. mayense* significantly increases the shoot length (19 cm) and shoot dry weight (235.67 mg) when compared with the un-inoculated control. However the inoculation of ICC1 results in no significant change on the vegetative parameter of *C. cajan* plant. In agreement with the present study Costa *et al*. (2014) also reported the characterization of rhizobia from *C.cajan* plant growing in Brazilian pantanal, which significantly increases the vegetative parameter of their host plants (Costa *et al*., 2014).

In case of *C. pallida* isolates, RCP2 and KACP2 which were identified as *M. jarvisii* successfully from root nodule on their host plant *C. pallida*. However, the other 2 isolates *viz.* DCP1 and NCP1 which were identified as *B. megaterium* and *B.koreensis* unable to form root nodule on their host plant. It was also found that inoculation of NCP1 significantly increases the shoot dry weight (8.44 mg) of *C. pallida* plant when compared with un-inoculated control (10.26 mg). The findings of this study are in accordance with those of López- Bucio *et al*. (2007), who found that *B. megaterium* isolated from the rhizosphere of maize plants enhanced the growth and development of host plant (Lopez-Bucio *et al.*, 2007). In nodulation test all the rhizobial isolates obtained from *M. pudica* (DMP2, SMP2, HMP1 and KMP1) formed nodules on their host plant. It was also revealed that inoculation KMP1 which was identified as *M. loti* significantly increases shoot dry weight (4.83 mg) of *M. pudica* plant when compared with un-inoculated control (2.41 mg). However, the remaining 3 isolates and reference strain *R. leguminosarum* were unable to induce any significant effect on the vegetative parameter of *M. pudica* plant. Similar to the present findings Klonowska *et al*. (2012), reported the characterization of rhizobia associated with *M. pudica* plant which significantly increases different vegetative parameters of host plant (Klonowska *et al*., 2012).