CHAPTER – 5

Discussion

5. DISCUSSION

Numerous bacterial strains were isolated both from the Rhizosphere as well as the root nodules of the source legume plants growing in the four valley districts of Manipur, India. Bacterial isolates from the rhizosphere of *Crotalaria juncea, Crotalaria pallida, Sesbania sesban, Leucaena leucocephala,* and *Parkia roxburghii* were isolated using the nitrogen free Burk's media (Wilson and Knight, 1952) in conformation with the work done by Park et al., (2005). The collection of the soil sample was carried out during the months between August-October e.i., during the end of monsoon and post monsoon period. Organisms isolated conforms to previously documents species such as *Bacillus, Enterobacter, Beijerinckia, Cedecea, Klebsiella and Pseudomonas.*

Bacterial isolates obtained from the root nodules of *Crotalaria juncea, Crotalaria pallida, Sesbania sesban* and *Leucaena leucocephala* were isolated using the nitrogen free YEMA medium in conformation with the work done by Zhang et al., (1991). Isolation of bacteria from *Parkia roxburghii* was omitted as there are no nodules present in the root. Collection of the root nodules sample was also carried out during the months between August-October *e.i.*, during the end of monsoon and post monsoon period. Again the organisms isolated conforms to previously documents species such as *Bacillus, Enterobacter, Mesorhizobium, Rhizobium, Pantoea, Cedecea, Bradyrhizobium, Stenotrophomonas and Pseudomonas.*

Collection of samples of both rhizosphere soil and root nodules during the August-October period was done in accordance with the fact that microbial growth were predominant during the said period due to high moisture content and nitrogen content in the soil as was described by Shilpkar et al., (2009). The present work gives more inclination to isolation and identification of the isolates through molecular characterisation, however some morphological and biochemical test were nevertheless performed in order to put more weightage to their characterisation.

5.1. Rhizosphere Isolates:

Twenty-six bacterial strains isolated from the rhizosphere of *Crotalaria juncea, Crotalaria pallida, Sesbania sesban, Leucaena leucocephala,* and *Parkia roxburghii* by using the nitrogen free Burk's media were chosen for further morphological and biochemical test. Amplification of 16S rDNA gene and sequence analysis were performed in accordance with the result obtained from ARA and nifH gene amplification. Preliminary morphological and

biochemical characterization were performed for all the isolates as per microbiological practical protocols described by Dubey *et al*., (2002). Twelve bacterial strains were selected after *Nif*H gene amplification for further characterization through 16S rDNA gene sequencing and phylogenetic analysis using bioinformatics tools.

Four bacterial isolates obtained from *Crotalaria pallida viz*., CPS.B-1, CPS.B-2, CPS.T-1 and CPS.IW-3 put these strains into three different family of organism after 16S rDNA analysis. The stains CPS.IW-3 positioned itself as *Bacillus subtilis*, CPS.B-1 as *Beijerinckia fluminensis* and both CPS.B-2 and CPS.T-1 positioned themselves as *Enterobacter asburiae*. 16S rDNA analysis of two bacterial isolates form *Sesbania sesban* conformed to two different organisms. Isolate SS.T conforms to *Enterobacter cloacae* and the isolate SS.IW-3 has been established as the organism *Cedecea davisae* in accordance with the result suggested after 16S rDNA analysis and NCBI Blast. Analysis of 16S rDNA sequences of two isolates from *Leucaena leucocephala i.e.,* LLS.T-1 and LLS.IE(B)-1 positioned themselves as the organisms *Enterobacter kobei* and *Bacillus altitudinis* respectively. Three isolates from the source plant *Parkia roxburghii viz.,* PS.IW-1, PS.IE-2 and PS.T(B)-2 which indicated some nitrogenase activity and also presence of NifH gene were also further determined through 16S rDNA analysis. The isolate PS.IW-1 got positioned as *Bacillus subtilis*, PS.IE-2 as *Klebsiella oxytoca* and the isolate PS.T(B)-2 conformed itself to the organism *Pseudomonas cedrina.* Only one isolate from *Crotalaria junceae,* CJS.IE-1 was found conforming to *Cedecea davisae* after 16S rDNA analysis. The isolate was selected for the same analysis even as it does not indicate any nitrogenase activity, however it produced a good band on NifH amplification.

The isolates *Bacillus subtilis* (CPS.IW-3 and PS.IW-1) shows an irregular colony of size of 2-4mm with an undulate margin, umbonate elevation and and white pigmentation. The isolate was observed to be Gram positive and possess motility also which is in agreement to Gordon et al., (1973); Buchanan et al, (1974). The isolate shows positive results for nitrate reduction, starch hydrolysis citrate utilization and catalase test which conforms to the result obtained by O'Donnell et al, (1980). The ARA result of the strain indicated its capability for nitrogenase activity hence suggesting nitrogen fixing capacity. The ability to fix nitrogen by *Bacillus subtilis* can further be supported by the presence of NifH gene as was suggested by Nhu and Diep, (2014). Already a work on the characterization of nitrogen fixing *Bacillus subtilis* strain was carried out by Satapute et al, (2012). Different documentation on isolation of *Bacillus subtilis* from different plant rhizosphere have been done separately by many like

Kumar et al, (2012); Gajbhiye et al, (2010); Islam et al, (2016). Moreover, isolation of *Bacillus* also has been reported from rhizosphere of various crop plants by Chan et al., (1994). Hence, isolation of the organism from the rhizosphere of *Crotalaria pallida* and *Parkia roxburghii* is in agreement with the previous works done by different individuals.

The organism *Bacillus altitudinis* obtained after 16S rDNA analysis of isolate showed a circular colony of size of 2-3mm with an entire margin, convex elevation and white pigmentation. The isolate was observed to be Gram positive and possess motility also which is in conformation with the work done by Habibi et al., (2014) and Shivaji et al., (2006). This strain was first isolated from cryogenic tubes used for collecting air samples from altitudes by Shivaji et al., (2006). Presence of nitrogenase activity shown by the isolate after ARA confirmed its ability to fix nitrogen as was reported on a work furnished by Habibi et al., (2014). The report is further supported by the amplification of NifH gene in the present work. Habibi et al., (2014) again gave the first report about *Bacillus altitudinis* being in association with rice plants and fix nitrogen.

The analysis of 16S rDNA sequences of two isolates from rhizosphere of *Crotalaria pallida* and one isolate each from the rhizosphere of *Leucaena leucocephala* and *Sesbania sesban* positioned the isolates among organisms from the *Enterobacteriaceae* family. The isolates designated as CPS.T-1 and CPS.B-2 were established as the organism *Enterobacter asburiae.* The isolate LLS.T-1 conformed to *Enterobacter kobei* whereas the isolates SS.T is being established as *Enterobacter cloacae.* All the four *Enterobacter* isolates showed a round or circular colony of 2-3mm in size, flat or raised with irregular edges and nonpigmented, which is in accordance with the details for *Enterobacteriaceae* family given in Bergey's Manual of Determinative Bacteriology, 9th edition (Holt et al., 1994). Except the isolate CPS.B-2, the other three isolates gives significant indication for nitrogenase activity as supported by the results of ARA. This finding is in agreement with the work done by Uchino et al, (1984) which reported that acetylene reduction activity of lenticellate warts of mangrove trunk bark is due to the presence of *Enterobacteriaceae.* Thokchom et al, (2013) described *Enterobacter hormaechei* as a rhizobacteria available in the rhizosphere of Mandarin orange having multiple PGP attributes including the capacity of nitrogen fixation. Isolation of *Enterobacter cloacae* from the rhizosphere of wetland rice has bee already reported in a work carried out by Fujie et al., (1987). Isolation of nitrogen fixing *Enterobacter cloacae* associated with sugar-cane and rice plants have already been reported (Rennie et al, 1982; Ladha et al, 1983). Amplification of NifH gene further supported and indicated the ability of these organisms for nitrogen

fixation. It is well known that many members of the *Enterobacteriaceae* family are capable of fixing nitrogen. In the present study, the isolation of organisms of *Enterobacteriaceae* family is in agreement with these findings.

16S rDNA analysis of isolate CJS.IE-1 from the rhizosphere of *Crotalaria pallida* and isolate SS.IW-3 from the rhizosphere of *Sesbania sesban* conforms with the organism *Cedecea davisae*, which is an extremely rare genus of the family *Enterobacteriaceae* (Janda and Sharon, 2006). The genus *Cedecea davisae* does not resemble any other group of the family *Enterobacteriaceae* according to a report furnished by Grimont et al, (1981). Despite many genera of *Enterobacteriaceae* are reported to be associative and endophytic diazotrophs, the same has not been reported about the organism *Cedecea davisae.* In the present study, both the isolates CJS.IE-1 and SS.IW-3 failed to show any significant amount of nitrogenase activity after ARA and multiple bands were also observed after NifH gene amplification. Therefore the nitrogen fixing ability of the organism remains inconclusive until further study.

The isolate CPS.B-1 from the rhizosphere of *Crotalaria pallida* positioned the strain and identified it as *Beijerinckia fluminensis* after 16S rDNA analysis. A preliminary analysis performed by Ruschel and Döbereiner, (1965) on the existence of nitrogen-fixing bacteria with grass showed that Beijerinckia spp. are the predominant bacteria in the rhizosphere. Moreover, Berkum and Bohlool, (1980) described Beijerinckia spp. as one of the most prominent among the genera occurring in the rhizosphere of a variety of tropical grasses. The strain *Beijerinckia fluminensis* obtained from the rhizosphere of Crotalaria pallida are associative bacteria having small, irregular and slimy colony of 1-3mm in size, flat or raised with irregular edges and light brown in colour, which is in accordance with the report given earlier by Döbereiner and Ruschel (1958). The authors also reported *Beijerinckia fluminensis* as one of the many associative and asymbiotic diazotrophs capable of nitrogen fixation. A report by Baldani and Baldani, (2005) also mentioned *Beijerinckia fluminensis* as one of the rhizospheric diazotrophs with graminaceous plants capable of nitrogen fixation. These findings are in agreement with the findings in the present work.

The sequences from PS.IE-2 positioned the strain and identified as *klebsiella oxytoca* after 16S rDNA analysis. *Klebsiella oxytoca* a gram negative non-motile facultatively anaerobic bacteria has already been documented as capable of fixing atmospheric nitrogen as determined by Acetylene Reduction technique and by protein content of cells (Lutfu Cakmakci *et al*, 1981). This previous report is supported by the result obtained after performing ARA in

the present study. Moreover it has been reported as an important microorganism for nitrogen fixation and chemical production (Guanhui Bao *et al*, 2013). An investigation by Arnold et al., (1988) on genetics of nitrogen fixation was initially revealed in *Klebsiella oxytoca*, reporting that nif genes necessary for synthesis of functional nitrogenase are clustered in a 24 kb region. Another work was reported about the isolation of *Klebsiella oxytoca* as an endophytic diazotroph from sweetpotato stems in Japan (Adachi et al, 2002). Isolation of *Klebsiella oxytoca* from the rhizosphere of Parkia roxburghii has never been reported before. However, its isolation from the rhizosphere of wetland rice has been already reported in a work carried out by Fujie et al., (1987).

The isolate PS.T(B)-2 obtained from the rhizosphere of *Parkia roxburghii* conformed with the organism *Pseudomonas cedrina* after 16S rDNA analysis. There are reports by Chan et al, (1994); Minkwitz and Berg, (2001); Vessey, (2003) on isolation of *Pseudomonas* from rhizosphere of various crop plants and nitrogen fixing strains also have already been reported for *Pseudomonas* (Dobereiner, 1989). However, no report of nitrogen fixation by *Pseudomonas Cedrina* have been documented so far. These findings of the bacterium *Pseudomonas Cedrina* is in contradiction with the result of ARA in the present study which revealed significant amount of nitrogenase activity. This result is again not in agreement with the amplification of NifH gene where it reveals multiple band. Consequently, the ability to fix nitrogen by *Pseudomonas Cedrina* cannot be ascertain during the present study.

5.2. Root Nodule Isolates:

Twenty bacterial strains isolated from the root nodules of *Crotalaria juncea*, *Crotalaria pallida, Sesbania sesban* and *Leucaena leucocephala* by using the nitrogen free YEMA media were chosen for further morphological and biochemical test. Amplification of 16S rDNA gene and sequence analysis were performed in accordance with the result obtained from ARA and nifH gene amplification. Preliminary morphological and biochemical characterization were also performed for all the isolates as per microbiological practical protocols described by Dubey *et al*., (2002). Fourteen bacterial strains were selected after *Nif*H gene amplification for further characterization through 16S rDNA gene sequencing and phylogenetic analysis using bioinformatics tools.

Four bacterial isolates obtained from the root nodules of *Crotalaria pallida viz*., CPN.B, CPN.IW-1, CPN.T-1 and CPN.T-4 put these strains into four different family of organism after 16S rDNA analysis. The isolate CPN.B conformed to the organism *Enterobacter hormaechei,*

CPN.IW-1 as *Stenotrophomonas maltophilia,* CPN.T-1 as *Pantoea agglomerans* and isolate CPN.T-4 positioned itself as *Cedecea davisae*. 16S rDNA analysis of three bacterial isolates form the nodules of *Sesbania sesban* conformed to three different organisms. Isolate SN.T conforms to *Mesorhizobium huakuii,* the isolate SN.IE-1 as *Pseudomonas azotoformans* and isolate SN.B-1 has been established as the organism *Neorhizobium huatlense* in accordance with the result suggested after 16S rDNA analysis and NCBI Blast. Analysis of 16S rDNA sequences of three nodules isolates from *Leucaena leucocephala i.e.,* LLN.B-1, LLN.T-1 and LLN.IE-1 positioned themselves as the organisms *Bacillus toyonensis, Pseudomonas hibiscicola* and *Mesorhizobium plurifarium* respectively. Four isolates from the nodules of *Crotalaria juncea* were choosen for 16S rDNA analysis which produced three different organisms. Isolates CJN.T-1 and CJN.B-1 were found to conformed with the organism *Pantoea agglomerans,* isolate CJN.IW-1 with *Bacillus subtilis* and the isolate CJN.IE-2 got positioned as the organism *Bradyrhizobium japonicum.*

16S rDNA analysis of CPN.B established the isolate as belonging to the organism *Enterobacter hormaechei*. As previously described, several of the genus belonging to *Enterobacteriacea* family are capable of nitrogen fixation. The statement is supported by the result obtained after ARA as well as the amplification of NifH gene. Thokchom et al, (2013) described *Enterobacter hormaechei* as a rhizobacteria available in the rhizosphere of Mandarin orange having multiple PGP attributes including the capacity of nitrogen fixation. Moreover, *Enterobacter hormaechei* has been reported as an endophytic bacterium of *Shorea selanica* which are compatible in supporting the growth of soybean (Widowati et al, 2013). These findings are in agreement with the findings in the present work.

The isolate CPN.IW-1 has been found to conform with the organism *Stenotrophomonas maltophilia.* Several strains of *Stenotrophomonas maltophilia* have been reported to be isolated from the rhizosphere and endosphere of various plants (Hayward et al, 2010). Preliminary characterisation of the isolate physiochemically and biochemically revealed the strain to be gram negative motile rod, testing positive for catalase and oxidase test whereas negative for citrate test which is in conformation with the work done by Park et al, (2005). The isolates revealed nitrogenase activity according to the result obtained from ARA. Furthermore, the ability for nitrogen fixation was supported by the amplification of NifH gene. These findings in the current work is in agreement with the findings reported by Reinhardt et al, (2008) and Teixeira et al (2007). Isolation of *Stenotrophomonas* from the nodules of *Vicia angustifolia* has been reported by (Kan et al, 2007). In a work reported by Reinhardt et al, (2008),

Stenotrophomonas maltophilia was isolated from the leaves and roots of cassava indicating the endophytic attributes of the organism.

16S rDNA analysis of the three different isolates CPN.T-1, CJN.T-1 and CJN.B-1 established the isolates as belonging to the same organism *Pantoea agglomerans* which is a Gram-negative bacterium that belongs to the family *Enterobacteriaceae*. All the three isolates showed a round or circular colony of 2-5mm in size, more or less convex with entire margins and yellowish in colour, which is in accordance with the details for *Pantoea agglomerans* given in Bergey's Manual of Determinative Bacteriology, 9th edition (Holt et al., 1994). Many reports about *Pantoea agglomerans* are available describing the organism as a common endophytes in root nodules (Kan et al., 2007) and in other plant tissues (Asis and Adachi, 2004; Burch and Sarathchandra, 2006). In the present study, the capacity of nitrogen fixation was determined by as well as nifH gene amplification. Isolates CPN.T-1 and CJN.B-1 revealed some amount of nitrogenase activity whereas the isolate CJN.T-1 showed a negligible amount of nitrogenase activity. The low amount of ethylene obtained for the isolate CJN.T-1 may be due to enzymatic activity or the culture conditions. The ARA result for the isolates revealed their capacity of nitrogen fixation which is in accordance with the findings obtained by Veena (1999) and Naiker (2003). Nitrogen fixing ability of the isolates were further supported by the amplification of NifH gene. In an investigation by Feng et al, (2006), an isolate of *Pantoea agglomerans* was described as a rice endophyte that promotes host plant growth and affects allocations of host photosynthates. Loiret et al, (2004) reported about a nitrogen fixing endophytic *Pantoea spp.* isolated from sugarcane stem tissue.

16S rDNA analysis of isolate CPN.T-4 from the root nodules of *Crotalaria pallida* conformed to the organism *Cedecea davisae,* which is an extremely rare genus of the family *Enterobacteriaceae* (Janda and Sharon, 2006). As described previously (see Rhizosphere isolates), the attributes of associative and endophytic diazotrophy have not been reported, despite many family of *Enterobacteriaceae* have been reported to be associative and endophytic diazotrophs. However, in the present study, contradictory to the isolates CJS.IE-1 and SS.IW-3, the isolate CPN.T-4 showed significant nitrogenase activity according to ARA and the amplification of NifH gene was also achieved. This contradiction in the results for the same organism may be due factors like contamination of the pure culture or the culture during ARA as well as the degradation and spoilage of the PCR product. Therefore the nitrogen fixing ability of the organism *Cedecea davisae* remains inconclusive until further study.

16S rDNA analysis of isolate SN.T from the root nodules of *Sesbania sesban* conformed to the organism *Mesorhizobium huakui.* that was first isolated from a winter-growing green manure crop, *Astragalus sinicus*, in Hubei, China by Huakui Chen (Chen and Shu, 1944). Isolate SN.T showed a circular and slimy colony of 2-4mm in size, raised or convex elevation with entire margins and yellowish in colour, which is in accordance with the details given for the organism *Mesorhizobium huakui* by Chen et al, (1991). All the families are known to be the most efficient nitrogen fixers in symbiosis with legumes. The ARA result and the NifH gene amplification in the present study supported this statement even if the result from the ARA is quite low. This may be due to the fact that *Mesorhizobium spp.* naturally have variations in their capacity of nitrogen fixation and adaptation to prevailing environmental stresses as documented by many previous works (Zharan, 1999; Maâtallah et al., 2002; L'taief et al., 2007). Isolation of the organism *Mesorhizobium huakui* from the nodules of *Sesbania sesban* in the present study is in agreement with many previous works (McInroy *et al*., 1999; Bala *et al*., 2002; Odee *et al*., 2002; Vinuesa *et al*., 2005) which reported that a variety of rhizobia *Mesorhizobium* spp. may induced *Sesbania* nodules.

16S rDNA analysis of isolate SN.IE-1 from the root nodules of *Sesbania sesban* conformed to the organism *Pseudomonas azotoformans* which is a gram negative bacterium which infects cereal grains, particularly rice (Iizuka and Komogata, 1963). The organism has been placed in the group of *Pseudomonas fluorescens* in accordance with 16S rRNA analysis (Anzai et al, 2000). Isolate SN.IE-1 showed a circular colony of 2-4mm in size, flat elevation with lobate margins and greenish in colour and shows motility which in accordance with the findings put forward by Iizuka and Komogata, (1963). *Pseudomonas azotoformans* is thought to be a nitrogen fixing organism according to Xie et al, (2006). Moreover, it has been reported as anitrogen fixing organism by Cui et al, (1996). These statements are supported by nitrogenase activity shown by the isolate after ARA even the amount is small and also by the amplification of NifH gene in the present study.

The isolate SN.B-1 has been found to conform to the organism *Neorhizobium huautlense* after 16S rDNA analysis. This gram negative root nodule nitrogen fixing bacteria was first isolated from *Sesbania herbacea* by Wang et al, (1998). The attribute of nitrogen fixation by *Neorhizobium huautlense* is supported in the present work by the result of ARA even if the amount is small and the amplification of NifH gene. The morphological characteristic of the isolate showing circular and slimy colonies, smooth margins, raised elevations and cream-coloured is in agreement with the findings reported by Wang et al,

(1998). So far there are no reports of isolation of *Neorhizobium huautlense* from *Sesbania sesban*, however species of Sesbania are known to form root nodules with fast growing rhizobia (Odee, 1990; de Lajudie et al., 1998b; Wang et al., 1998) which,could be any of the genera of Rhizobium, Mesorhizobium, Sinorhizobium, Azorhizobium or Allorhizobium (Young and Haukka, 1996; Wang and Martinez-Romero, 2000; Giller, 2001). Moreover, the findings of a work done by Bala et al, (2002) revealed that *Sesbania sesban* is nodulated by a much wider range of rhizobia than previously thought.

The isolate CJN.IW-1 from the root nodules of *Crotalaria junceae* has been found to conformed with the organism *Bacillus subtilis* after 16S rDNA analysis. As described previously in rhizosphere isolates, the bacterium *Bacillus subtilis* is a well-known nitrogen fixer as suggested by several previous works and also supported by the present study. The endophytic attribute of *Bacillus subtilis* have been reported in several investigations by different individuals such as by Lin et al, (2009); Gupta et al, (2015); Wang et al, (2009); Li et al, (2012). Moreover, *Bacillus subtilis* has been reported as an endophytic bacteria isolated from rice plant cultivated on soil of Phu Yen province, Vietnam in a work done by Nhu and Diep, (2014).

16S rDNA analysis of isolate CJN.IE-2 from the root nodules of *Crotalaria junceae* conformed with the organism *Bradyrhizobium japonicum* after 16S rDNA analysis. This is a species included in a group of legume-root nodulating, microsymbiotic nitrogen fixing bacteria. *Bradyrhizobium* have been reported to nodulate *Crotalaria* which are well inhabited in tropical and warm areas in both hemispheres by Samba et al, (1999); Sy et al, (2001). So far there are no accurate report about isolation of *Bradyrhizobium japonicum* from *Crotalaria junceae,* however isolation of strains of broad-host-range *Bradyrhizobium sp.* from *Crotalaria sp.* have already been reported (Van Rossum et al, 1995; Samba et al, 1999). An investigation carried out by Aserse et al, (2012) also reported about the isolation of phylogenetically diverse groups of *Bradyrhizobium* from the nodules of different species of *Crotalaria*. All these findings support the findings of the present work which reveals that the isolation of *Bradyrhizobium japonicum* from the nodules of *Crotalaria junceae* as a valid one.

The isolate LLN.B-1 from the root nodules of *Leucaena leucocephala* has been found to conform to the organism *Bacillus toyonensis* after 16S rDNA analysis. *Bacillus toyonensis,* a novel novel species of the *Bacillus cereus* group was isolated in 1966 in japan. In the present study, the isolates showing a gram positive, motile rod with colonies of 2-3mm, entire edges,

flat elevations with a creamy pigmentation is in accordance with the findings reported by Jimenez et al, (2013). Several species of *Bacillus* are known nitrogen fixers as mentioned previously, however there are no reports of nitrogen fixation by *Bacillus toyonensis* so far. The result of ARA in the present study indicates some nitrogenase activity but at the same time there was no amplification of NifH gene in the present study. The result of ARA may be due to the culture condition of the broth culture or contamination of the culture during the analysis. The capacity of nitrogen fixation by *Bacillus toyonensis* thus remained in question until further study.

16S rDNA analysis of isolate LLN.T-1 from the root nodules of *Leucaena leucocephala* conformed to the organism *Pseudomonas hibiscicola* after 16S rDNA analysis. Isolation of Pseudomonas have been reported from rhizosphere of various crop plants (Chan et al, 1994; Minkwitz and Berg, 2001; Vessey, 2003) and nitrogen fixing strains also have already been reported for *Pseudomonas* (Dobereiner, 1989). However, no report of nitrogen fixation by *Pseudomonas hibiscicola* have been documented so far. These findings of the bacterium *Pseudomonas hibiscicola* is supported by the present study in accordance with the lack of nitrogenase activity after ARA and the amplification of multiple band during NifH gene amplification.

The isolate LLN.IE-1 from the root nodules of *Leucaena leucocephala* has been found to conform to the organism *Mesorhizobium plurifarium* after 16S rDNA analysis. Diverse rhizobial groups of *Mesorhizobium* as well as *Rhizobium* and *Sinorhizobium* have been reported to nodulate *Leucaena leucocephala* in Mexican field (Wang et al, 1999). There are reports of isolation of the rhizobia *Mesorhizobium plurifarium* associating with *Leucaena leucocephala* from America, Asia, Africa and Australia (De Lajudie et al, 1998). *Leucaena* and *Sesbania* species are suggested to belonged to the same cross-nodulation group by Trinick (1980) which explained the isolation of *Mesorhizobium* from both these plants in the present study as a valid one.