CHAPTER 2
REVIEW OF LITERATURE

2. Review of literature

2.1. Taxonomy of Rhizobia

2.1.1. Earlier studies

In the year 1888, Beijerinck first reported the isolation of the root nodule bacteria and established that they were responsible for nitrogen fixation in legume plants (Beijerinck, 1888). In the following year, Frank, (1889) named the root nodule bacteria as *Rhizobium* with originally only one species, *Rhizobium leguminosarum* (Frank, 1889). Since then, a number of rhizobial species have been reported from diverse legume plants and the taxonomy of rhizobia has been changing from time to time due to the isolation of new rhizobial genera and species. In previous studies morphological characteristics, such as rod-shape, gram negative, aerobic and motile nature of the rhizobia were used as criteria for rhizobial classification. However, later it was found that these criteria's could not always distinguish rhizobia from other soil bacteria.

In the year 1932, Fred *et al*. proposed a new criteria known as cross inoculation group concept for rhizobial classification (Fred *et al*., 1932). This method classified the rhizobial genera based on their host plants. The method also distinguishes rhizobia from *Agrobacterium* since the genus *Rhizobium* contain species involved in nodule formation on legume roots, whereas, *Agrobacterium* comprised plant pathogenic species. Based on the cross inoculation concept the root nodule forming microbes were classified into 6 groups under one genus *Rhizobium* (Jordan & Allen, 1974). These 6 groups were classified into fast and slow growing rhizobial species based on their growth rate, DNA base composition and the genera of host plants. Rhizobia that nodulated *Pisum, Trifolium, Phaseolus* and *Medicago* were identified as fast growing *Rhizobium leguminosarum*, *Rhizobium trifolii, Rhizobium* *phaseoli* and *Rhizobium meliloti*, respectively, while 2 slow growing bacterial species that nodulated *Lupinus* and *Glycine* were identified as *Rhizobium lupini* and *Rhizobium japonicum,* respectively. The slow growing *Rhizobium japonicum* were later included into a new genus *Bradyrhizobium* (Jordan, 1982). Fast growing rhizobia have generation times of less than 6 hours and generally forms visible colonies on yeast extract mannitol agar media within 2-5 days; whereas slow growers have generation times of more than 6 hours and give detectable growth after more than 5 days under standard conditions. Most of the slow growing rhizobia produce alkali while fast growers produce acid (Jordan, 1984). The cross inoculation concept played a significant role in defining rhizobial species for a long time, because it was believed that all rhizobia could be classified according to their host ranges. However, in later years it was observed that legume species can be infected by more than one genetically different rhizobial species. In addition a single rhizobial species can nodulate more than one host legume. For example, *Rhizobium* sp. NGR234 was reported to nodulate more than 120 genera of legumes (Pueppke & Broughton, 1999).

2.1.2. Recent studies

From the 1980s on, with the introduction and advancement of a variety of DNA-based methods in the identification of rhizobia, more diversity was discovered among the rhizobia and their relationships with other groups of bacteria became apparent. Molecular methods like, DNA- DNA hybridization, PCR-RFLP analysis of ribosomal DNA, ribosomal DNA gene sequencing and symbiotic characteristics were considered as standard guidelines to describe new rhizobial species (Jarvis *et al*., 1997). The genotypic characterization of rhizobia provided more information on rhizobial diversity and substantially increasing the number of genera, species and strains which are known to be capable of producing effective nodules on legumes.

Rhizobial taxonomy has progressed notably in the last few decades, mainly due to the characterization of new isolates together with the use of 16S rDNA sequencing and polyphasic taxonomic approaches.

Earlier, rhizobia were believed to be composed of traditional alpha-proteobacteria or alpha-rhizobial strains. However, recent works on rhizobial diversity using advanced molecular techniques reported the isolation of many beta-proteobacteria or beta-rhizobial strains: *Burkhoderia, Cupriavidus* from different leguminous plants (Vandamme *et al*., 2002). According to Current Taxonomy of Rhizobia and List of Prokaryotic names with Standing in Nomenclature, rhizobial species described so far belong to 12 genera of alpha and 3 genera of beta-rhizobia (Laranjo *et al*., 2014). The 12 genera of alpha-rhizobia currently consist of 113 species: *Aminobacter* (1), *Azorhizobium* (3), *Bradyrhizobium* (15), *Devosia* (1), *Mesorhizobium* (29), *Methylobacterium* (1), *Microvirga* (3), *Ochrobactrum* (2), *Phyllobacterium* (1), *Rhizobium* (43), *Sinorhizobium*/*Ensifer* (13) *and Shinella* (1). The ability of beta-rhizobia to nodulate plants changed the view that bacteria of alpha-rhizobia genera only are able to nodulate legumes (Moulin *et al*., 2001; Chen *et al*., 2003). The 3 genera of beta-rhizobia currently consist of 9 species: *Burkholderia* (6), *Cupriavidus* (2) and *Herbaspirillum* (1). Further, there are large no of species of rhizobia that do not form nodules and do not fix nitrogen, but perform different functions in soil or may exist as an endophyte in plant tissues. Phylogenetic analysis of 16S rDNA, *nodA* and *nifH* genes from alpha and beta-proteobacteria suggests that beta-rhizobia have evolved from diazotrophs through multiple gene transfer.

2.1.3. Taxonomy of alpha-rhizobia

Rhizobium: *Rhizobium* is the most common and widely studied genera of rhizobia. In 1888, Beijerinck first reported the isolation of bacteria from root nodules and established that they were responsible for nitrogen fixation (Beijerinck, 1888). He named these bacteria as *Bacillus radicicola*. Later, Frank changed the name to *Rhizobium* with originally just 1 species, *R. leguminosarum* (Frank, 1889). The members of this genus are short gram negative rods, 0.5 to 0.9 µm wide and 1.2 to 3 µm in length. They are motile with polar flagella, aerobic and chemo-organotrophs. It currently consists of 43 species which includes *R. leguminosarum*, *R. tropici*, *R. etli*, *R. gallicum*, *R. mongolense*, *R.undicola*, *etc.*

Sinorhizobium: Chen *et al*. (1988) first proposed a separate genus for the fast growing soybean rhizobia, renaming *Rhizobium fredii* as *Sinorhizobium fredii* (Chen *et al*., 1988). The separation was based on phenotypic and genotypic features. Currently the species of the genus *Sinorhizobium* were transferred to the genus *Ensifer* since the latter is the synonym that takes the priority. The genus currently consists of 13 species. It includes the species *E. fredii*, *E. meliloti*, *E. terangae* and *E. sahelense*, *E. medicae etc*.

Mesorhizobium: The genus *Mesorhizobium* was described in 1997 (Jarvis *et al*., 1997), in order to reassign some species previously included in the *Rhizobium* genus. They are characterized by a growth rate intermediate between the fast and slow growing rhizobia. *Mesorhizobium* can establish nitrogen-fixing symbiosis with wide varieties of legume species. They are Gram-negative rods, motile by polar flagellum and aerobic in nature. It currently consists of 29 species which includes *M. loti*, *M. huakuii*, *M. ciceri*, *M. mediterraneum*, *M. tianshanense*, *etc*.

Bradyrhizobium: *Bradyrhizobium* was created for the slow growing species of rhizobia (Jordan, 1982). Originally the soybean nodulating *B. japonicum* was the only species described. They are slow growing in contrast to *Rhizobium* species, which are considered fast-growing rhizobia. *Bradyrhizobium* species take 3–5 days to create a moderate turbidity in a broth medium and 6–8 hours to double in population size. These are rods with a single polar or sub-polar flagellum. It currently consists of 15 species which include *B. japonicum*, *B. betae*, *B. elkanii*, *B. diazoefficiens*, *B. yuanmingense*, *etc*.

Azorhizobium: They are short rods very similar to *Rhizobium*, with polar and peritrichous ciliation, but produce an alkaline reaction on glucose as *Bradyrhizobium* (Dreyfus *et al*., 1988). Currently it consists of 3 species of symbiotic bacterium: *A. caulinodans, A. dodereinereae* and *A. oxalatiphilum*.

Allorhizobium: *Allorhizobium* genus was segregated from the genus *Rhizobium* (de Lajudie *et al*., 1998). Previous works reported that most of the species of *Allorhizobium* form endosymbiotic nitrogen-fixing association with roots of legumes, while few are known to cause crown gall.

In earlier studies rhizobia were thought to belong to only the 6 above mentioned genera of alpha-rhizobia viz. *Rhizobium, Azorhizobium*, *Allorhizobium, Mesorhizobium*, *Ensifer* (syn. *Sinorhizobium*) and *Bradyrhizobium*. However, with the development of advanced molecular biology techniques, new rhizobial species were identified from other genera of the alpha-proteobacteria. These included nitrogen fixing bacterial species in the genera *Devosia* (Rivas *et al*., 2002), *Methylobacterium* (Sy *et al*., 2001), *Ochrobactrum* (Trujillo *et al*., 2005), *Phyllobacterium* (Valverde *et al*., 2005), *Aminobacter* and *Shinella* (Lin *et al*., 2008).

2.1.4. Taxonomy of beta-rhizobia

Burkholderia: The *Burkholderia* genus has been proposed by Yabuuchi *et al*. in 1992 (Yabuuchi *et al*., 1992). They are gram negative, motile with one or more polar flagella. The first diazotrophic bacterial species within the genus *Burkholderia* was *B. vietnamiensis* (Gillis *et al*., 1995). The *Burkholderia* genus contains rhizospheric bacteria, human pathogenic bacteria, as well as nitrogen fixing bacteria. Different species of *Burkholderia* contain common nodulation genes (*nodABC*, *nodD*, *nifH*) that are very similar to standard rhizobial symbiotic genes. Therefore, it has been hypothesized that *Burkholderia* recruited symbiotic genes by horizontal gene transfer. This genus currently contains 6 species.

Cupravidus: In 2001, Chen *et al*. first reported, the isolation of *Ralstonia taiwanensis* from root nodules of *Mimosa pudica* and *Mimosa diplotricha* (Chen *et al*., 2001). In this case the strain was classified as *Ralstonia* and it has been later named *Cupriavidus taiwanensis*. Although several species of this genus were isolated from soil and human clinical specimens but *C. taiwanensis* was the only species able to form effective nodules and to fix atmospheric nitrogen in legumes.

2.2. Molecular methods used in rhizobial diversity and phylogenetic studies

Successful management of symbiotic associations between legumes and rhizobia requires the identification of specific strains. Traditional methods for distinguishing microbial strains were morphological, physiological and biochemical. Phenotypic features such as colony morphology, and physiological or biochemical responses of a bacterium may vary depending on the media and conditions used for growing it. However, these methods when applied to rhizobia, frequently fail to identify strains within a species. Examples of modern techniques include various molecular approaches such as studying bio-diversity among rhizobia through RAPD

(Williams *et al*., 1990), rep-PCR (Versalovic *et al*., 1991), DNA-DNA hybridization technique (Martens *et al*., 2008), RFLP or 16S rRNA gene sequencing using specific primers (Laguerre *et al*., 1994; Weisburg *et al*., 1991)and determination of *nod* and *nif* gene expression for nodulation and nitrogen fixation. Molecular genetic markers in general provide better information, more sensitive and relatively more accurate to study the relationship of closely related bacterial strains and in detection of higher rhizobial diversity than the phenotypic techniques.

The ribosomal ribonucleic acid (rRNA) gene is considered as a model genetic marker for studying the evolutionary history of life. Bacteria contain genes coding for 5S, 16S and 23S rRNAs in which all are typically structured into a segment of the genome, called rRNA operon. The rRNAs are vital components of ribosomes that are involved in translation of messenger RNA and protein synthesis (Acinas *et al*., 2004; Jensen *et al*., 1993). From last few decades the 16S rRNA gene sequence has been used as a standard genetic marker in identification and phylogenetic classification of prokaryotic microorganisms including rhizobia (Woese *et al*., 1990). This marker has several properties that make it suitable for phylogenetic inference. These are

A) 16S rRNA is universally found in all bacteria, this enables the comparison of the phylogenetic relationship of all organisms and allows the construction of a tree of life.

B) It is relatively highly conserved, thus the function of this gene over time has not changed, indicating that random sequence variation that may occur among organisms are more exact measure of evolution.

C) The bacterial 16S rRNA gene sequence is a long stretch (1500 bp) that includes both conserved and variable regions, which can offer enough information for taxonomic purpose.

Because of the above mentioned significance, 16S rRNA gene or 16S rDNA sequence has been used extensively as a main criterion for phylogenetic classification of prokaryotes. Generally, 97% 16S rDNA sequence similarity has been considered as a threshold for differentiating one species from other (Stackebrandt & Goebel, 1994). However, nowadays using 16S rRNA gene sequence for microbial diversity, phylogenetic and population studies must be regarded with caution as it was recognized that a bacterial genome can have multiple and heterogeneous ribosomal RNA operons (Acinas *et al*., 2004) and these can be also affected by horizontal gene transfer and genetic recombination (Eardly *et al*., 2005). In many of the rhizobial groups, this region has slight divergence between species (Vinuesa *et al*., 2005; Willems *et al*., 2003).

Besides these housekeeping genes, some additional genes are commonly included in the rhizobial species description. The most studied symbiotic genes of rhizobia are *nodD*, *nodA*, *nodC* and *nifH*. These genes are located either on plasmids or symbiosis islands in the chromosomes of the bacteria. In species of *Mesorhizobium* and *Rhizobium* housekeeping genes are found in the plasmids (Young *et al*., 2006; Wang *et al*., 1999). The symbiotic genes in *Mesorhizobium loti* are located on symbiotic islands in the chromosome flanked by insertion sequences (Kaneko *et al*., 2000). The analysis of these symbiotic genes is also useful to identify non *Rhizobium* species able to nodulate legumes.

2.3. Rhizobia diversity studies from India

India is a mega biodiversity center and studies of symbionts of legumes may unravel the presence of new types of bacterial groups in their nodules. Many new species of rhizobia are described each year from India, and even strains from non-typical rhizobia genera have been added to the rhizobia list.

2.3.1. Diversity of rhizobia associated with cultivated legumes of India

Most of the cultivated legumes can form symbiotic associations with rhizobia. Isolation and characterization of nitrogen fixing rhizobia from the nodules of cultivated leguminous plants have been reported from different regions of India. Deka & Azad, in 2006, isolated and investigated the diversity of rhizobia from 6 common pulses viz., cowpea, greengram, blackgram, pigeonpea, soybean and groundnut (Deka & Azad, 2006). They collected nodule samples from these plants from 6 different geographical sites, namely Guwahati, Tezpur, Lakhimpur, Nalbari, Hajo and Majuli. The study revealed that the isolates showed a wide variety in their morphological features such as colony margin, colony elevation and colony color. The isolates also showed variation in their pattern of carbohydrate utilization. Their study confirmed that most of the isolates belong to slow growing *Bradyrhizobium*. Likewise, in 2010, Dubey *et al*. isolated rhizobia from *Cajanus cajan* based on their physiological and biochemical characteristics using standard methods (Dubey *et al*., 2010). 16S rDNA gene sequencing analysis of the isolates revealed that they belong to the genera *Ensifer*. They confirmed the nodulation abilities and nitrogen fixing abilities of the isolates by amplification of *nodC* gene and *nifH* genes. Their study also revealed that the isolates were capable of IAA production and phosphate solubilization. Kundu *et al*. in 2008 isolated rhizobia from mung bean plants growing in 17 different locations of Haryana Agricultural University and authenticated by plant infection test (Kundu & Dudeja, 2008).

Soybean plant (*Glycine max* L.) is an important source of protein and is an important grain legume. Many previous studies reported the characterization of rhizobia from the soybean plant cultivated in different regions of India. In 2008, Appunu *et al*. studied genetic diversity of soybean nodulating *Bradyrhizobium* isolates from different agricultural regions of India (Appunu *et al*., 2008). They studied the diversity based on PCR-RFLP analysis of the 16S rRNA gene, the intergenic spacer region between the 16S and 23S rRNA genes, *nifH* and *nodC* genes. Their investigation revealed the presence of 3 different lineages among the soybean isolates which include *B. liaoningense* and *B. yuanmingense*. In 2010, Bikrol *et al.* also characterized *Bradyrhizobium* strains from soybean plants grown in an agricultural field of Sehore district of Madhya Pradesh (Bikrol *et al*., 2010). Their study revealed a considerable level of genetic diversity among the *Bradyrhizobium* isolates. In the same year, Sharma *et al*. (2010) isolated rhizobial isolates from 12 different soybean growing sites of Madhya Pradesh, India, for biochemical and metabolic characterization (Sharma *et al*., 2010). Based on carbon source utilization pattern they observed remarkable metabolic diversity among the rhizobial isolates. Recently, Ansari *et al*. (2014) characterized the diversity of soybean rhizobia from central India and analyzed them based on phenotypic features, such as utilization of carbon sources and intrinsic resistance to antibiotics (Ansari *et al*., 2014). The results of the study revealed that the slow growing soybean rhizobia comprised of *Bradyrhizobium* spp. and *Rhizobium radiobacter* strains, whereas the fast growing strains exclusively belonged to *R. radiobacter.*

Kamble *et al*. in 2006 characterized rhizobial isolates from root nodules of various chickpea cultivars grown in agricultural research fields and determined their enzymatic and antibiotic resistance abilities (Kamble *et al*., 2006). Rai *et al*. (2012) isolated rhizobial strains from chickpea plants collected from different regions of Peninsular and Northern India and analyzed them on the basis of phenotypic and genotypic traits (Rai *et al*., 2012). Their study revealed the presence of efficient salt tolerant strains which tolerated 1.5% NaCl and efficient phosphate solubilizing strains. In the following year, Yadab *et al*. (2013) isolated rhizobia from root nodules of chickpea plants grown at Mau district of Uttar Pradesh, India (Yadab *et al*., 2013). Their results revealed that the isolates belonged to different strains of *Mesorhizobium*, *Rhizobium*, *Bradyrhizobium* and *Agrobacterium* genera. In recent past, Saini *et al*. (2015) isolated bacteria from roots and nodules of chickpea grown in Haryana Agricultural University (Saini *et al*., 2015). Their study revealed that a large number of endophytes from the roots and nodules solubilized phosphate and produced ammonia. 16S rRNA gene sequencing analysis revealed that the isolate belongs to *Bacillus subtilis* and *Bacillus licheniformis*.

Appunu *et al*. (2011) isolated rhizobia from root nodules of horsegram collected from different regions of south India and analyzed them based on different phenotypic and genotypic features (Appunu *et al*., 2011). Based on the sequence analysis of 16S-23S rDNA IGS genotypes horsegram rhizobia were distributed into 5 divergent lineages of *Bradyrhizobium* genus. Likewise, in 2015, Edulamudi *et al*. characterized rhizobia from the root nodules of horsegram plants grown in different regions of Andhra Pradesh and Telangana states, India (Edulamudi *et al*., 2015). They characterized the isolates on the basis of RAPD and PCR-RFLP analysis. Their study revealed a wide genetic diversity among the isolates.

2.3.2. Diversity of rhizobia associated with wild legumes of India

Although most of the diversity related study of rhizobia was confined to cultivated legumes, few researchers have also studied the rhizobial diversity of few wild legumes. Pandey *et al.* (2004) investigated the diversity of rhizobia isolated from selected wild medicinal legumes from 2 different locations of India, Haridwar and Dehradun (Pandey *et al*., 2004). Their results revealed that the isolated strains belong to 2 different genotypes *S. meliloti* and *B. japonicum*. In the following year, Pandey *et al*. (2005) also reported the isolation bacterial strain which belongs to beta-proteobacteria from root nodules of *Mimosa pudica* (Pandey *et al*., 2005). They named the bacteria as *Burkholderia* sp. MSSP and investigated their plant growth promoting properties. The results of their study reported that the isolates can secretes phytohormone, ACC deaminase, solubilizes phosphate and antagonistic against phytopathogens. Arun *et al*. (2005) investigated the stress tolerance abilities of rhizobia isolated from 5 wild legume plants *Canavalia maritima* Thouars, *Crotalaria retusa* L., *Canavalia cathartica* Thouars, *Crotalaria verrucosa* L. and *Derris triflorum* DC from the dunes of Someshwara and Padubidri, Mangalore, India (Arun & Sridhar, 2005). They reported that rhizobial isolates obtained from these wild plants showed optimum growth in a temperature range of 30–40 ^{0}C, can tolerate up to 4.5% NaCl and showed optimum growth at acidic pH (5.0 – 6.0). In the following year, Kumar *et al*. (2006) investigated the genetic diversity of rhizobia from a medicinal legume, *Mucuna pruriens* grown in Haridwar, India (Kumar *et al*., 2006). Their study revealed that the isolates solubilized inorganic phosphate and produced IAA. In 2009, Elanchezhian *et al*. isolated rhizobia from root nodules of *Vigna marina* (Burm.) Merr. collected from the seashores of Andaman and Nicobar Islands, India (Elanchezhian *et al*., 2009). They reported that the rhizobial isolates were tolerant to different antibiotics like erythromycin, chloramphenicol, streptomycin, ampicillin and showed resistance up to 600 mM of NaCl.

Kumari *et al*. (2010) studied nodulation characteristics in 5 species of *Indigofera* viz., *I .trita*, *I. linnaei*, *I. astragalina*, *I. parviflora and I. viscosa* (Kumari *et al*., 2010). The results revealed that the isolates showed catalase, nitrate reductase, tryptophanase and citrate activity while variation was observed for enzymes like caseinase, lipase, amylase, lysine decarboxylase, gelatinase, urease and protease activities. Dhabhai *et al*. (2012) isolated and characterized 5 rhizobia isolates from the woody legume tree, *Acacia nilotica* L. (Dhabhai *et al*., 2012). 16S rRNA gene sequencing analysis of the isolates revealed that 1 isolate showed strong homology with *Mesorhizobium loti* and the other isolate showed intermediate homology with *Rhizobium leguminosarum* and *Rhizobium hainanense*. Rasul *et al*. (2012) isolated rhizobial strains nodulating *Millettia pinnata*, collected from southern regions of India (Rasul *et al*., 2012). The results showed high phenotypic and genetic diversity among the rhizobia. The results of 16S rRNA gene sequence analysis revealed that most microorganisms are closely related to *B. elkanii*, *B. yuanmingense* and *Rhizobium sp*. Among the isolates, some could grow in a pH range of 4.0 – 10.0, some could tolerate a high salt concentration (3% NaCl) and could grow at a maximum temperature between 35 °C and 45 °C. They have characterized the isolate on the basis of phenotypic, phylogenetic distinctiveness and molecular data. In a recent study, Bhargaba *et al*. (2016) investigated morphological, biochemical, stress tolerance and plant growth promoting characteristics of rhizobial isolates from the nodules of wild legumes growing in Tirupati, India (Bhargava *et al*., 2016). The bacterial isolates were confirmed as rhizobia based on morphological features and biochemical tests. The isolates are tolerant up to 1% NaCl, displayed normal growth at temperatures 28˚C - 30˚C, and showed remarkable growth at neutral pH and poor growth at pH 5 and 9.

2.4. Rhizobial diversity studies from selected legume plants

Legumes have been used in agriculture by the farmers since ancient times. Legume seeds were among the first source of human food and their cultivation in many areas occurred at the same time as that of the major cereals. Because of the fixed nitrogen legume species grow better than otherplants in poor, degraded soils and they tolerate environmental stress better than other plants. *Leguminosae* is the third largest family of flowering plant after *Orchidaceae* and *Asteraceae*. It consists of 16,000- 19,000 species in about 750 different genera (Allen & Allen, 1981). The family *Leguminosae* is categorized into 3 subfamilies: *Caesalpiniaceae*, *Mimosaceae* and *Fabaceae*. Previous reports suggested that most species of the *Leguminosae* family form symbiotic associations with nitrogen fixing rhizobia. It was also reported that 97% of the *Papilionoideae*, 90% of the *Mimosoideae* and 23% of the *Caesalpinioideae* were found to have symbiotic associations with nitrogen fixing rhizobia (Sprent, 1995). Legume plants also form symbiotic associations with arbuscular mycorrhizal fungi which help them in taking up nitrogen and phosphorus from the soil (Sprent, 2001). In recent past years, efforts have been made to study the diversity and population structure of rhizobia nodulating cultivated herbaceous legumes, as well as wild legume trees, shrubs and medicinal plants in various regions of the world (Khbaya *et al*., 1998; Li *et al*., 2008).

In the present study four legume plants are selected for isolation of indigenous rhizobial strains. These include two cultivated legume plants, namely *Lablab purpureus* L. and *Cajanus cajan* L. and two wild legume plants, namely *Crotalaria pallida* L. and *Mimosa pudica* L.

2.4.1. Previous studies on characterization of rhizobia from *Lablab purpureus* L.

Lablab purpureus L. was believed to have originated in India and is widely distributed in many countries where it has become naturalized (Deka & Sarkar, 1990). It grows in various types of soils, ranging from acid to alkaline soils (pH 4.4-7.8). In last few years isolation and characterization of rhizobia from *L. purpureus* plants received a considerable attention. Previously, rhizobia of *L. purpureus* plant were classified as the cowpea miscellany (Allen & Allen, 1981), a heterogeneous group of slow growing rhizobia. However, the exact taxonomic position of slow-growing rhizobia associated with *L. purpureus* has not been clarified because the genus *Bradyrhizobium* was established a little later in 1982 (Jordan, 1982) and, although 9 valid species, as well as many invalid species have been described since then, there are only very few reports to date concerning the diversity of rhizobia associated with *L. purpureus*.

2.4.2. Previous studies on characterization of rhizobia from *Cajanus cajan* L.

C. cajan or pigeon pea plant can form a symbiotic association with a large diversity of indigenous rhizobia in soil. The slow and fast growing pigeon pea rhizobia present great genetic and metabolic diversity (Ramsubhag *et al*., 2002; Fernandes Júnior *et al*., 2012). In addition to being efficient in fixing nitrogen in field conditions, pigeon pea rhizobia also present other biotechnological applications and enzymatic activity (Fernandes Júnior *et al*., 2010). Datta & Basu, (2000) isolated *Rhizobium* spp. from root nodule of *C. cajan* plant and revealed that the isolates were capable of producing a very high amount of IAA (Datta & Basu, 2000). Some studies also reported the stress tolerant abilities of rhizobia isolated from *C. cajan* root nodules (Fernandes Júnior *et al*., 2012). Recently, Costa *et al*. (2014) isolated rhizobial isolates from root nodule of *C. cajan* collected from Brazilian Pantanal and investigated their stress tolerance abilities (Costa *et al*., 2014).

2.4.3. Previous studies on characterization of rhizobia from *Crotalaria pallida* L.

Crotalaria is one of the largest genera of *Fabaceae* with more than 500 species commonly found in diverse climatic regions (Allen & Allen, 1981).Specific reports about rhizobia associated with *Crotalaria* spp. are veryfew. The only information about the rhizobia was obtained from a few studieson tropical isolates which included only a few strains from *Crotalaria* spp. Inthese studies rhizobial strains isolated from *Crotalaria* were designated as *Bradyrhizobium* spp.(Gao *et al*., 1994). Liu *et al*., 2007, characterized rhizobia from *C. pallida* grown in tropical and subtropical regions of China based on their phenotypic and genotypic features (Liu *et al*., 2007).

2.4.4. Previous studies on characterization of rhizobia from *Mimosa pudica* L.

Like other *Mimosa* species, *M. pudica* most commonly interacts with rhizobia belonging to the beta-proteobacteria, which are now termed as beta-rhizobia (Moulin *et al*., 2001; Gyaneshwar *et al*., 2011). In 2004, Verma *et al*. (2004) isolated and characterized bacteria from the nodules of *M. pudica* from North India and South India (Verma *et al*., 2004). Their study revealed that most of the *M. pudica* isolates were closely related to *R. taiwanensis*. Likewise, Pandey *et al.* (2005) also isolated root nodule harboring *Burkholderia* strains from *M. pudica* and determined their plant growth promoting abilities (Pandey *et al*., 2005). Although beta-rhizobia are the major symbionts of *Mimosa pudica*, some alpha-rhizobia strains were also isolated from the root nodules of rhizobia (Liu *et al*., 2011).