Biotechnology and Molecular Biology Review Vol. 2 (2), pp. 049-057, June 2007
Available online at http://www.academicjournals.org/BMBR
ISSN 1538-2273 © 2007 Academic Journals

Mini Review

Non-rhizobial nodulation in legumes

D. Balachandar*, P. Raja, K. Kumar and SP. Sundaram

Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore – 641 003, India

Accepted 27 February, 2007

Legume - Rhizobium associations are undoubtedly form the most important N_2-fixing symbiosis and play a subtle role in contributing nitrogen and maintaining/improving soil fertility. A great diversity in the rhizobial species nodulating legumes has been recognized, which belongs to α subgroup of proteobacteria covering the genera*, Rhizobium, Sinorhizobium* (renamed as *Ensifer*), *Mesorhizobium, Bradyrhizobium* and *Azorhizobium*. Recently, several non-rhizobial species, belonging to α and β subgroup of Proteobacteria such as *Methylobacterium, Blastobacter, Devosia, Phyllobacterium, Ochrobactrum, Agrobacterium, Cupriavidus, Herbaspirillum, Burkholderia* and some γ-Proteobacteria have been reported to form nodules and fix nitrogen in legume roots. The phylogenetic relationship of these non-rhizobial species with the recognized rhizobial species and the diversity of their hosts are discussed in this review.

Key words: Legumes, Nodulation, Proteobacteria, Rhizobium

Table of content

INTRODUCTION

Members of the leguminosae form the largest plant family on earth with around 19,000 species (Polhill et al., 1981). The success of legumes can largely be attributed to their ability to form a nitrogen-fixing symbiosis with specific bacteria known as rhizobia, manifested by the development of nodules on the plant roots in which the bacteria fix atmospheric nitrogen, a major contributor to the global nitrogen cycle. Symbiosis between legumes and rhizobia are of considerable environmental and agricultural importance since they are responsible for most of the atmospheric nitrogen fixed on land (Graham and Vance, 2003). Among the 19,000 species described so far, only a small proportion has been studied for their nodulation ability. The legume biodiversity is concentrated in tropical regions, while most studies are on cultivated leguminous plants from temperate region (Moulin et al., 2001).

Rhizobia are able to elicit symbiotic association on most of the species of leguminosae, forming nodules in roots in which they reduce atmospheric nitrogen to ammonia for the benefit of plant. Nodule formation is controlled by extracellular bacterial signal molecules, called

*Corresponding author. E-mail: dbalu2000@yahoo.com.

nod factors, which are recognized by the host plant (Lerouge et al., 1990; Schultze and Kondorosi, 1998). The rhizobial species described so far are very diverse and do not form an evolutionary homologous clade. They are ∝ subgroup Proteobacteria, belonging to three distinct branches which are phylogenetically intervened with many non-symbiotic bacteria (Young and Haukka, 1996; Zakhia and deLajudie, 2001). The first large branch groups the genera *Rhizobium, Sinorhizobium,* (renamed as *Ensifer*), *Mesorhizobium* with *Agrobacterium,* a pathogenic bacterium of plant. The second branch contains the genus *Bradyrhizobium* and the third branch includes the genus *Azorhizobium*. Each rhizobial species has a defined host range, varying from very narrow to very broad. Apart from rhizobia, very close relatives of rhizobia such as members of ∝ subgroup of proteobacteria and few β-Proteobacteria are also recognized to form nodules in legumes, which are the focus of this review.

Non-rhizobial nodulations

In the past five years, several symbionts capable of forming nodules and fixing nitrogen in legume roots have been documented and grouped under ∝ and β subclass of Proteobacteria, which include *Methylobacterium nodulans* (Sy et al., 2001), *Blastobacter denitrificans* (Van Berkum and Eardly, 2002), *Devosia* sp.(Rivas et al., 2002), *Ochrobactrum lupini* (Trujilo et al., 2005), *Agrobacterium* like strains (Mhamdi et al., 2005), *Phyllobacterium trifolii* (Valverde et al., 2005) , *Herbaspirillum lustianum* (Valverde et al., 2003), *Ralstonia taiwanensis* (renamed as *Cupriavidus taiwanensis*) (Chen et al., 2001), *Burkholderia tuberum, Burkholderia phymatum* (Vandamme et al., 2002), *Burkholderia cepacia* (Rasolomampianina et al., 2005) and few γ-proteobacteria (Benhizia et al., 2004). The legume host preferred by these non-rhizobial proteobacteria possesses high diversity.

∝ Proteobacteria

Methylobacterium

The genus *Methylobacterium* includes a variety of pink pigmented facultative methylotrophic bacteria (PPFMs) that are able to grow on C1 compounds such as formate, formaldehyde and methanol as sole carbon source, as well as on wide range of multi-carbon growth substances (Green, 1992). *Methylobacterium* belongs to ∝-Proteobacteria, comes under order Rhizobiales and family Methylobacteriaceae. Recently, Sy et al. (2001) reported *M. nodulans*, a facultative methylotrophic species forming nodules in the legume, *Crotalaria* spp., the only species of that genus able to form nodules and also fix nitrogen. It was also confirmed that this species harbors the nodulating genes, *nodABC* and genes encoding structural nitrogenase enzyme by PCR amplification (Sy et al., 2001; Jourand et al., 2004). Phylogenic analysis of *M. nodulans* shows it is closer relationship to other species of *Methylobacterium* (Sy et al., 2001), whereas, the phylogenic tree based on *nifH* gene shows that it is more related to *Gluconacetobacter diazotrophicus,* a sugarcane endophyte of ∝-Proteobacteria (Figure 1). *M. nodulans* type strain ORS2060 is able to form nitrogen fixing nodules in *Crotalaria glaucoides, Crotalaria perrottetii* and *Crotalaria podocarpa*. Similarly, another legume, *Lotonois bainesii* was also reported to be a host for nodulation and nitrogen fixation by pink pigmented *Methylobacterium* (Jaftha et al., 2002). Raja et al. (2006) isolated nodulating and non-nodulating *Methylobacterium* sp. from legumes with high nitrogenase activity. Similarly, Mathaiyan et al. (2006) isolated several nodulating *Methylobacterium* from tropical legumes such as field beans, cowpea, black gram, soybean, *Sesbania* with high nitrogenase activity. These isolates are able to form effective nodules in *Crotolaria juncea*. Their *nif* and *nod* gene diversity and mode of infection are to be examined.

Blastobacter

Blastobacter spp. is commonly aquatic (especially freshwater) budding bacteria that form rosette structures by cellular attachment to a common base. They belong to ∝-Proteobacteria, order Rhizobiales and family Brady-rhizobiaceae. The flood tolerant leguminous plant, *Aschynomene indica* is most frequently nodulated by *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* (Van Berkum et al., 1995). Van Berkem and Eardly (2002) isolated *B. denitrificans* capable of forming an effective nitrogen fixing symbiosis with *A. indica*. They are able to form effective nodules in the roots of *A. indica* and reduce the atmospheric nitrogen (11.5 µM of C_2H_4 produced per plant as ARA) (Table 1), which was again confirmed by presence of *nifHDK* genes by southern hybridization. The predicted results revealed that *B. denitrificans* could well be a symbiotic bacterium of *A. indica,* which has very close phylogenetic relation with *Bradyrhizobium* spp (Figure 2). Further confirmation of presence of *nif* gene diversity of this species is essential to reclassify this bacterium as *Blastobacter* or renaming the genera as *Bradyrhizobium*.

Devosia

The α-Proteobacterium, *Devosia,* currently includes a known species *Devosia riboflavina,* isolated from soil. This species is Gram-negative, aerobic, non-sporulated motile rod that contains long-chain 3-hydroxy fatty acids. Recently, isolates from nodules of aquatic legume *Neptunia natans* from India were reported to be phylogenetically very close to *D. riboflavina* (95.9 % similarity), but not the same species (Rivas et al., 2002).

Devosia is in the family Hyphomicrobiaceae within the order Rhizobiales of α-Proteobacteria. The other rhizobia of this family, *Azorhizobium caulinodans* does not show

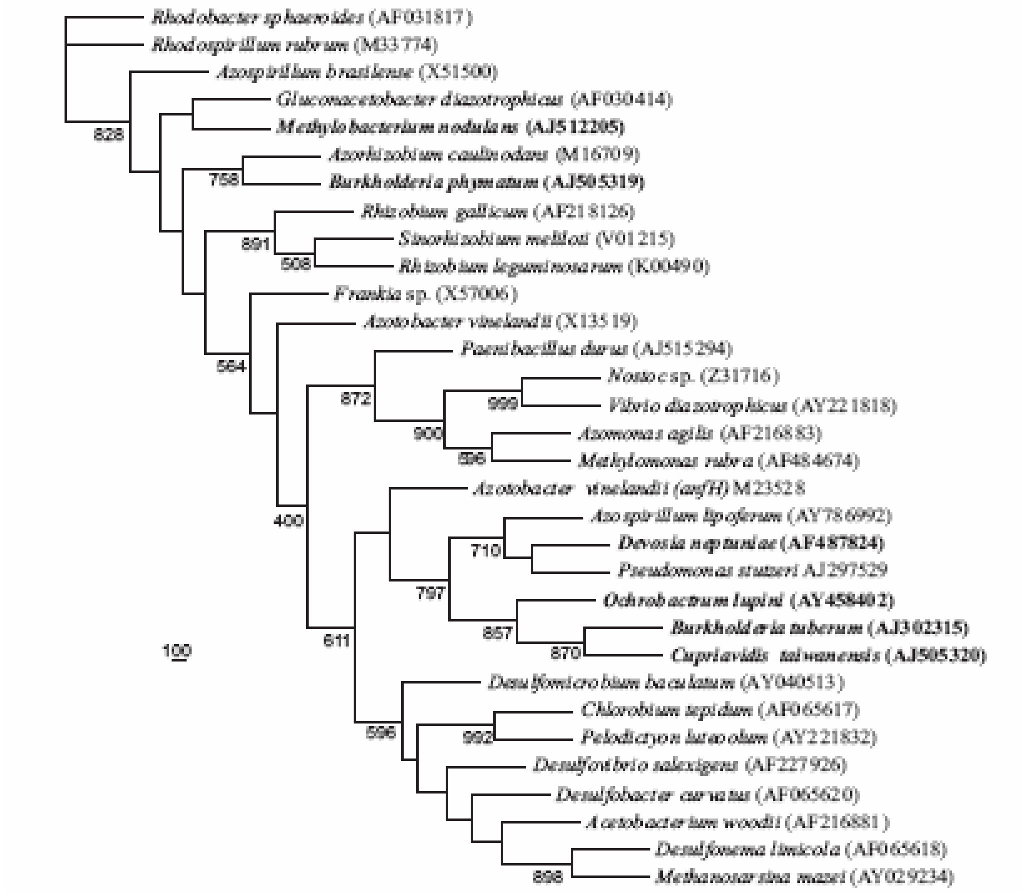


Figure 1. Phylogenetic tree based on a 368 bp fragment of *nifH* gene showing relationship of non-rhizobial nodulating proteobacteria with other nitrogen fixing bacteria. *nifH* sequences (accession number in parentheses) obtained from GenBank were used for phylogenetic tree construction. Phylogenetic tree was constructed by neighbor-joining method using DNADIST, SEQBOOT, NEIGHBOR and CONSENSE from PHYLIP v3.5c and the tree file was analysed using tree view. Bootstraps values of 500 or more (from 1000 replicates) are indicated at the nodes. The *nifH* of non-rhizobial proteobacteria are highlighted by bold letters.

Table 1. Nodulation and nitrogen fixation of non-rhizobial Proteobacteria in their respective legume roots.

Proteobacteria	No. of nodule /plant	Nitrogenase activity[a]	Reference
Blastobacter denitrificans (LMG8443^T)	NR	11.5	Vab Berkum and Eardly (2002)
Burkholderia sp. (Br3454^T)	19.0	2.91	Chen et al. (2005)
Ochrobactrum lupini (LUP21^T)	7.0	NR	Trujillo et al. (2005)

[a] Acetylene reduction assay in nmol of ethylene produced /h/plant, NR – Not reported

close relatedness, as far as 16S rDNA phylogeny is concerned. But, *nodD* gene of *Devosia* spp. nodulating *N. natans* is closely related to *Rhizobium leguminosarum* (Figure 3a). The presence of *nod* and *nif* genes were detected in *Devosia* isolates by PCR amplification, which are located in 170 kbp sized plasmids (known as *sym* plasmids) as that of most of the fast growing rhizobia. The species nodulating *N. natans* was named as *Devosia neptuniae* (Rivas et al., 2003).

Phyllobacterium

Phyllobacterium sp. is a soil inhabitating proteobacteria; belong to the family Phyllobacteriaceae of order Rhizobiales, having recognized species, *Phyllobacterium myrsinacearum* (Mergaert et al., 2002). *Phyllobacterium* species was originally proposed to accommodate bacteria isolated from leaf nodules of Rubiaceae and Myrsinaceae tropical plants (Knosel et al., 1984).

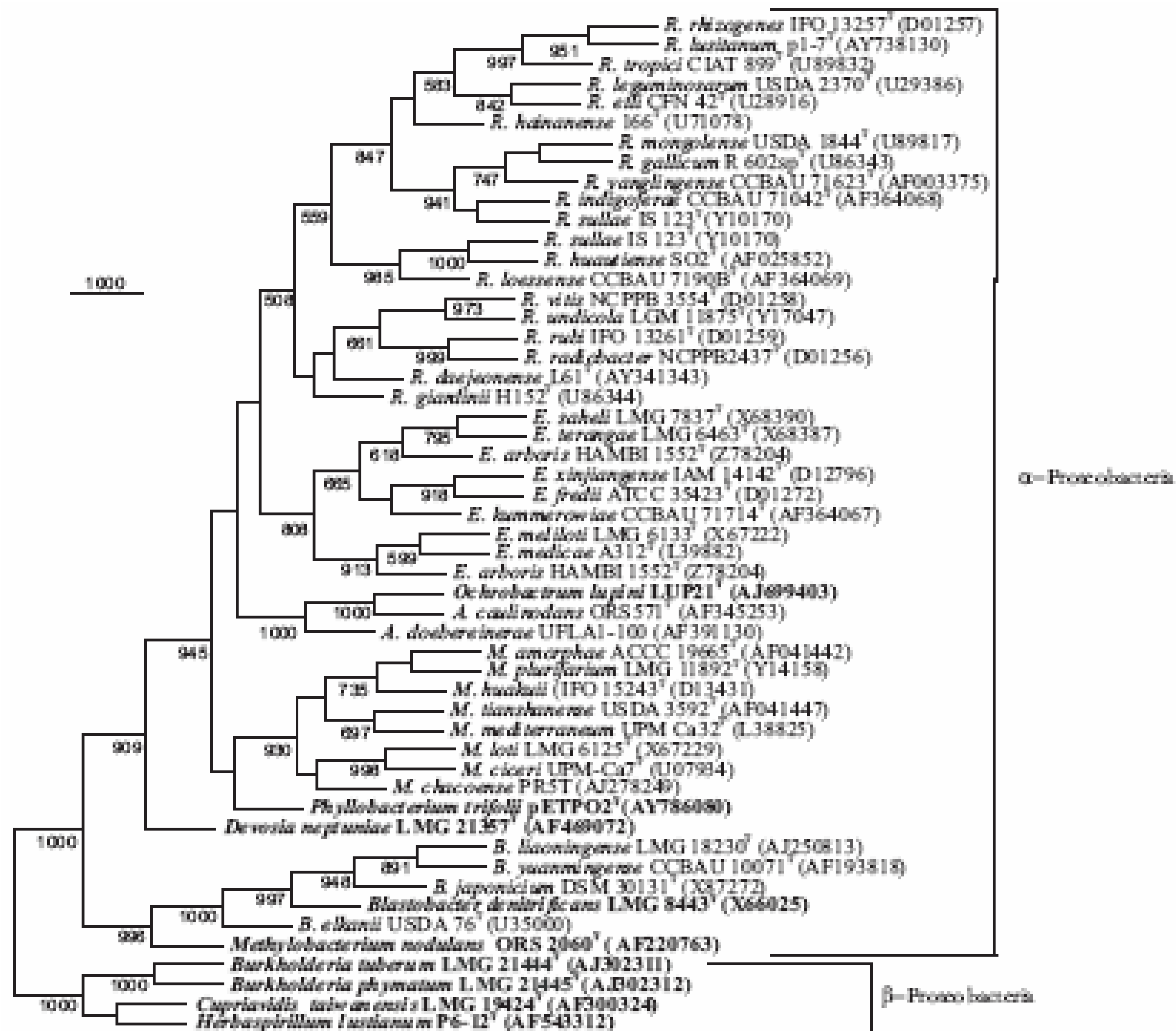
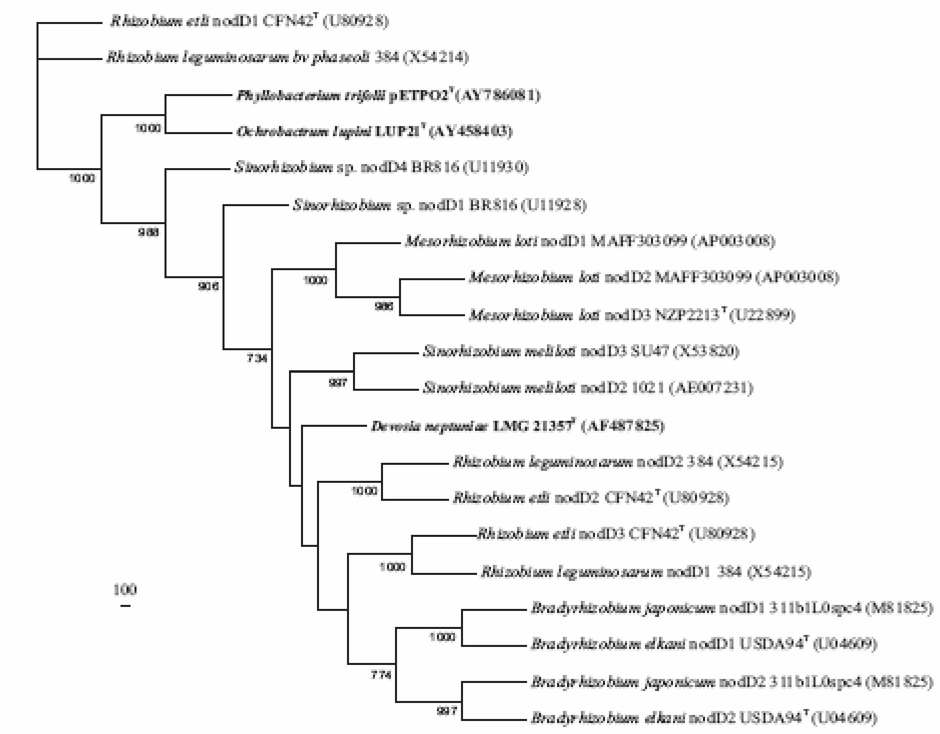


Figure 2. Phylogenetic relationship of rhizobia and other nodulating Proteobacteria based on 16S rRNA gene sequence comparisons. The sequence of type strain of representative rhizobial species and non-rhizobial species obtained from GenBank were used for phylogenetic tree construction. Sequence accession number of 16S rRNA of type strains are given in parentheses. Phylogenetic tree was constructed by neighbor-joining method using DNADIST, SEQBOOT, NEIGHBOR and CONSENSE from PHYLIP v3.5c and the tree file was analysed using tree view. Bootstraps values of 500 or more (from 1000 replicates) are indicated at the nodes.

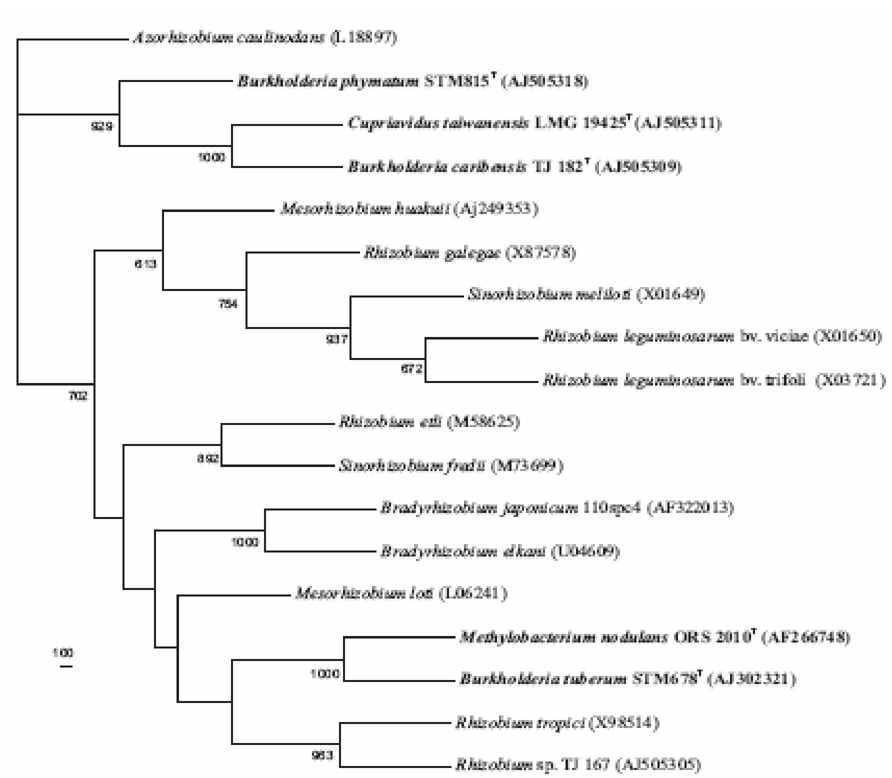
Recently, another species of *Phyllobacterium*, *P. trifolii* was isolated from nodules of *Trifolium pratense* growing in Spanish soil (Valverde et al., 2005). Plant infection experiments with this species (Strain pETPO2^T) revealed that it forms effective nodules in the roots of *Trifolium repans* and *Lupinus albus*. The 16S ribosomal gene sequence comparative analysis revealed more closeness towards *P. myrsinacearum* (98 % similarity) and the closest rhizobia, *Mesorhizobium* spp (Figure 2). The species, *P. trifolii* differs with *P. myrsinacearum* both in cultural characters as well as the fatty acid methyl ester (FAME) profile. The sequence of *nodD* gene is more similar to other non-rhizobial nodule forming β-Proteobacterium, *Ochrobactrum*, (94.9 % similarity) which forms nodules in *Lupinus* than rhizobial species (Figure 3a). The plasmid profile revealed that *P. trifolii* harbors symbiotic plasmids in which nodulating and nitrogen fixing genes are located.

Ochrobactrum

Ochrobactrum, a α_2 subgroup of Proteobacteria belonging to the family Brucellaceae and order Rhizobiales, was first described by Holmes et al. (1988) with a single species, *Ochrobactrum anthropi*. Later four different species were recognized from different ecological origins, clinical sources, soil, wheat rhizosphere and chicken faeces.



(a)



(b)

Figure 3. Comparative sequence analysis of *nodD* (a) and *nodA* (b) genes from non-rhizobial Proteobacteria and rhizobia. Sequence data obtained from GenBank were used for phylogenetic tree construction by neighbor-joining method using DNADIST, SEQBOOT, NEIGHBOR and CONSENSE from PHYLIP v3.5c and the tree file was analysed using tree view. Bootstraps values of 500 or more (from 1000 replicates) are indicated at the nodes. The non-rhizobial proteobacteria are highlighted by bold letters and sequence accession numbers are in parentheses.

Recently Ngom et al. (2004) isolated *Ochrobactrum* strain from root nodules of *Acacia mangium*. Trujilo et al. (2005) isolated a novel nodulating species, *O. lupini* from *Lupinus honoratum.* The 16S rRNA phylogeny showed that the strains of *O. lupini* do not belong to the rhizobial genera described until now. The nodulating species, *O. lupini* strain LUP23^T is more closely related to other species of *Ochrobactrum,* namely *O. anthropi, O. tritici* etc. *O. lupini* strain LUP21 and LUP23 harbor the mega plasmids of size 1500, 200 and 150 kbp. The nodulating and nitrogen fixing genes (*nod* and *nif* genes) were detected in all the *sym* plasmids using *nifH* and *nodD* probes. The isolate is able to form nitrogen fixing nodules in *L. albus* and this is the only species of Brucellaceae capable of nodule formation in legume, *Lupinus*.

Agrobacterium

Bacteria related to *Agrobacterium* were also identified among the root nodules of several tropical leguminous plants including *Acacia, Prosophis* and *Chamaecrista* from Africa (De Lajudie et al., 1999). Similarly, a significant portion of bacterial isolates of *Phaseolus vulgaris* grown in Tunisian soils has 16S rRNA genes similar to those of *Agrobacterium tumifaciens* and *Agrobacterium rubi* (Mhamdi et al., 2002). No symbiotic and nitrogen fixing genes were detected by PCR amplification of *nifH* and *nodC* genes and hybridization of total genomic DNA with nitrogen fixing gene probes. Mhamdi et al. (2002) confirmed that these *Agrobacterium* like strains entered the nodules by mixed infection with a rhizobial cell capable of nodule induction resulting mixed population within the nodule. When *Agrobacterium* cells mixed with rhizobia, (*R. gallicum*), it was able to occupy about 10-15 % of the total nodules in *P. vulgaris*, indicating that agrobacteria are likely to be confused as one of the non-rhizobial nodulations in legumes, while isolated from such broad hosts.

β Proteobacteria

Cupriavidus

Cupriavidus is a β-Proteobacteria, belonging to family Burkholderiaceae of order Burkholderiales. Several species of this genus were isolated from soil and human clinical specimens (Vandamme and Coeyne, 2004). Recently, *C. taiwanensis* (originally reported as *Ralstonia taiwanensis* or *Wautersia taiwanensis*) was isolated from nodules of *Mimosa pudica* and *Mimosa diplotricha* (Chen et al., 2001). Among many species of *Cupriavidus*, *C. taiwanensis* was the only species able to form root nodules in legumes. Phylogenetically, *C. taiwanensis* is distinct to all the rhizobial species (Figure 2) and are very close to other species of *Cupriavidus*. This species forms effective nodules in *M. pudica* and *M. diplotricha* and fixes atmospheric nitrogen. Further, two *sym* plasmids with approximate size of 3.5 and 2.4 Mb were identified in *C. taiwanensis*, in which *nod* and *nif* genes are presented (Chen et al., 2003).

Herbaspirillum

Herbaspirillum, a nitrogen fixing soil bacterium able to establish close associations with plants especially the grass plants, through epiphytic colonization in rhizoplanes and endophytic colonization in roots (Oliveres et al., 1997). It belongs to β-Proteobacteria, coming under the family Burkholderiaceae of order Burkholderiales. Recently a new species, *H. lustitanum* was isolated from root nodules of *P. vulgaris* from Portugal soils (Valverde et al., 2003). Complete 16S rDNA sequence based phylogenetic analysis of the isolate revealed that *H. lusitanum* is closely related to *Herbaspirillum seropedicae* and *Herbaspirillum rubrisubalbicans*. Further, this nodulating species is differentiated from other species by ARDRA (Amplified ribosomal DNA restriction analysis) and TP-RAPD (Two primer randomly amplified polymorphic DNA) patterns (Valverde et al., 2003). The presence of *sym* plasmids and host specificity of nodulation are to be evaluated for further establishment.

Burkholderia

Burkholderia, a β subclass Proteobacteria belonging to family Burkholderiaceae of order Burkholderiales, is a common soil habitant bacterium, often associated with plant roots. Few endophytic species were also recorded in maize. Moulin et al. (2001) isolated *Burkholderia* sp. from nodules of South African legume, *Aspalathus carnosa.* The 16S ribosomal DNA sequence homology revealed that it is very close to the species *Burkholderia kururiensis* (96.9 % identity). The isolate is able to form nodules in the roots of *Macroptilium atropurpureum,* a tropical legume, capable of establishing diverse rhizobial symbiosis. Further, the presence of *nodABC* through PCR confirmed that it is capable of producing nod factors to initiate the nodulation. Similarly, nodulating and nitrogen fixing *Burkholderia* species, namely *Burkholderia caribensis, B. phymatum* and *B. tuberum* were isolated from *Mimosa bimucronata* and *Mimosa pigra* (Vandamme et al., 2002; Chen et al., 2003; Chen et al., 2005). All these isolates are able to form effective nodules in *M. pudica, Mimosa diplotricha, M. pigra* and *Mimosa acutishpula* with nitrogenase activity (Table 1). Sequence similarity analysis of *nodA* showed that *B. phymatum* is closer to other non-rhizobial nodulating bacterium, *Cupriavidus taiwanensis,* whereas *B. tuberum* is close to *nodA* of *Methylobacterium nodulans* (Figure 3b).

γ-Proteobacteria

Benhizia et al. (2004) examined the nodules of three Mediterranean wild legume species, *Hedysarum carno-*

Table 2. The α- and β-Proteobacteria, nodulating legumes and their taxonomical position.

Non-rhizobial species	Host legume	Phylogenetic group	Taxonomical position		Reference
			Family	Order	
Methylobacterium nodulans	*Crotalaria glaucoides*	α-Proteobacteria	methylobacteriaceae	Rhizobiales	Sy et al. (2001)
Blastobacter denitrificans	*Aschynomene indica*	α-Proteobacteria	Bradyrhizobiaceae	Rhizobiales	Van Berkum and Eardly(2004)
Devosia neptuniae	*Neptunia natans*	α-Proteobacteria	Hyphomicrobiaceae	Rhizobiales	Rivas et al. (2002)
Phyllobacterium trifolii	*Trifolium repans*	α-Proteobacteria	Phyllobacteriaceae	Rhizobiales	Valverde et al. (2005)
Ochrobactrum lupini	*Lupinus albus*	α-Proteobacteria	Brucellaceae	Rhizobiales	Trujilo et al. (2005)
Agrobacterium like strains	*Phaseolus vulgaris*	α-Proteobacteria	Rhizobiaceae	Rhizobiales	Mhamdi et al. (2005)
Burkholderia tuberum, *B. phymatum*	*Mimosa*	β-Proteobacteria	Burkholderiaceae	Burkholderiales	Vandamme et al. (2002)
Cupriavidus taiwanensis	*Mimosa*	β-Proteobacteria	Burkholderiaceae	Burkholderiales	Chen et al. (2001)
Herbaspirillum lusitanum	*Phaseolus vulgaris*	β-Proteobacteria	Burkholderiaceae	Burkholderiales	Valverde et al. (2003)

sum, Hedysarum spinosissimum subsp. *capitatum* and *Hedysarum pallidum* and identified very high diversity among the microsymbionts nodulating these wild legumes. ARDRA profiles and RAPD fingerprinting analysis of these microsymbionts revealed that nodules of these wild legumes are not formed due to single species of rhizobia and surprisingly include many gamma Proteobacteria *viz., Enterobacter kobei, Enterobacte cloaceae, Leclercia adecarboxylata, Pantoea agglomerans, Escherichia vulneris* and *Pseudomonas* sp. No evidence of any rhizobial like sequence was found even upon amplifying from bulk of microbial cells obtained from squashed nodules, suggesting that exclusive occupants are members of the order Enterobacteriales or Pseudomonadales, which are belonging to gamma Proteobacteria (Bennizia et al., 2004). This is the first report of gamma Proteobacteria associated with legume nodules. Presence of *nifH, nodA, nodD* genes and their divergence in these γ-Proteobacteria need to be examined to include these bacteria as rhizobia.

Conclusion

Rhizobia are wide spread soil bacteria able to induce the formation of root nodules and to fix nitrogen on cultivated and wild legumes. These rhizobia are of economic importance in low input sustainable agriculture, agroforestry and land reclamation. Currently there are five genera of rhizobia in α-Proteobacteria recognized as *Azorhizobium, Bradyrhizobium, Rhizobium, Meso-rhizobium* and *Ensifer* (Zakhia and deLajudie, 2001). Besides these genera, several α- and β-Proteobacteria and several unclassified γ-Proteobacteria were recently isolated from nodules of diversified legume plants as summarized in Table 2. Several reports as summarized in this review are available about the occurrence of new species of Proteobacteria with the ability to nodulate specific host legumes. Most of these Proteobacteria nodulating legumes are characterized based on the genotypic, phenotypic and chemotaxonomic data and designated as new species. The *nifH* and *nodD* or *nodA* gene sequence divergence was also reported for few of these isolates. These approaches demonstrate the need for more mole-cular studies on:

i. Interaction between these Proteobacteria with their legume host, in terms of signal exchange.
ii. Diversity of flavonoids and nod factors which regulate the nodulation process.
iii. Mode of entry and infection process of these Proteobacteria in legumes.
iv. Interrelation between these Proteobacteria and with rhi-zobia in terms of *nif* and *nod* genes.
v. Source of *nif* and *nod* genes made these bacteria to form nodules (Perret et al., 2000).

These studies are to be addressed for better understanding the "origin of nodules" and for enhancing the nodulation and nitrogen fixing efficiency of these new group of Proteobacteria.

Nitrogen fixation, which is wide spread in Eubacteria and Archaea, is thought to be an ancestral function now lost by many bacteria. Conversely, nodulation is thought to have appeared in evolution, at the same time as the appearance of legumes on earth, about 70 -130 million years ago. At this period of history, α- and β-Proteobacteria and different rhizobial linkages already had diverged. The genes required for legume nodulation are thought to have been acquired subsequently by lateral transfer from undefined sources, thus converting soil saprophytes into symbionts (Hirsch et al., 2001). This hypothesis has been confirmed by many recent works (Sullivan and Ronson, 1998) and presence of very similar and phylogenetically related *nodABC* genes in nodulating α- and β-Proteobacteria strongly supports the hypothesis of a unique origin of *nod* genes. However, it is not clear whether a single transfer event was responsible for the spread of nodulation genes from one subclass to the other.

The wide spread character of nodulation by many Proteobacteria, dominated by rhizobia is also attested by very high diversity of legumes. Since many legumes and environments remain to be explored, it is likely that further characterization of rhizobia from new legumes will reveal an even greater diversity.

REFERENCES

Benhizia Y, Benhizia H, Benguedouar A, Muresu R, Giacomini A, Squartini A (2004). Gamma Proteobacteria can nodulate legumes of the genus *Hedysarum*. Syst. Appl. Microbiol. 27: 462 – 468.

Chen WM, deFaria SM, Straliotto R, Pitard RM, Simoes-Araujo JL, Chou JH, Chou, YJ, Barrios E, Prescott AR, Elliott GN, Sprent JI, Young JPW, James EK (2005). Proof that *Burkholderia* strains form effective symbioses with legumes: a study of novel *Mimosa* nodulating strains from South America. Appl. Environ. Microbiol. 71: 7461-7471.

Chen WM, Laevens S, Lee TM, Coenye T, Vos PD, Mergeay M, Vandamme P (2001). *Ralstonia taiwanensis* sp. nov., isolated from root nodules of *Mimosa* species and sputum of cystic fibrosis patient. Int. J. Syst. Evol. Microbiol. 51: 1729 – 1735.

Chen WM, Moulin L, Bontemps C, Vandamme P, Bena G, Masson CB (2003). Legume symbiotic nitrogen fixation by β Proteobacteria is wide spread nature. J. Bacteriol. 185: 7266-7272.

De Lajudie P, Willems A, Nick G, Mohamed SH, Torck U, Coopman R, Filali-Maltouf A, Kersters K, Dreyfus B, Lindstrom K, Gillis M (1999). *Agrobacterium* bv. 1 strains isolated from nodules of tropical legumes. Syst. Appl. Microbiol. 22: 119-132.

Graham PH, Vance CP (2003). Legumes: Importance and Constraints to Greater Use. Plant Physiol. 131: 872-877.

Green PN (1992). The genus *Methylobacterium* In A. Balows, H.G. Truper M, Dworkin M, Harder W, Schleifer KH (eds) The Prokaryotes, Springer – Verlag, New York. pp. 2342-2349.

Hirsch AM, Lum MR, Downie JA (2001). What makes the rhizobia-legume symbiosis so special? Plant Physiol. 127: 1484-1492.

Holmes B, Popoff M, Kiredjian M, Kersters K (1988). *Ochrobactrum anthropi* gen. nov., sp. nov., from human clinic specimens and previously known group Vd. Int. J. Syst. Bacteriol. 38: 406-416.

Jaftha JB, Strijdom BW, Steyn PL (2002). Characterization of pigmented methylotrophic bacteria which nodulate *Lotononis bainesii*. Syst. Appl. Microbiol. 25: 440-449.

Jourand P, Girand E, Bena G, Sy A, Willems A, Gillis M, Dreyfus B, deLajudie P (2004). *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. Int. J. Syst. Evol. Microbiol. 54: 2269-2273.

Knosel DH (1984). Genus IV. *Phyllobacterium* nom. rev. In: Krieg NR, Holt JG, (eds) Bergey's Manual of systematic Bacteriology Vol. 1, Williams and Wilkins, Baltimore, pp. 254-256.

Lerouge P, Roche P, Faucher C, Maillet F, Truchet G, Prome JC, Denarie J (1990). Symbiotic host-specificity of *Rhzobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. Nature 344: 781-784.

Madhaiyan M, Poonguzhali S, Senthilkumar M, Sundaram SP, Sa T (2006). Nodulation and plant growth promotion by methylotrophic bacteria isolated from tropical legumes. Microbiol. Res. (On line Publ.)

Mergaert J, Cnockaert MC, Swings J (2002). *Phyllobacterium myrsinaecearum* (Subjective synonym *Phyllobacterium rubiacearum*) emend. Int. J. Syst. Evol. Microbiol. 52: 1821-1823.

Mhamdi R, Laguerre G, Aouani ME, Mars M, Amarger N (2002). Different species and symbiotic genotypes of field rhizobia can nodulate *Phaseolus vulgaris* in Tunisian soil. FEMS Microbiol. Ecol. 41: 77-84.

Mhamdi R, Mrabet M, Laguerre G, Tiwari R, Aouani ME (2005). Colonization of *Phaseolus vulgaris* nodules by *Agrobacterium* like strains. Can. J. Microbiol.51:105-111.

Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2001). Nodulation of legumes by members of the β subclass of Proteobacteria. Nature 411: 948-950.

Ngom A, Nakagawa Y, Sawada H, Tsukahara J, Wakabayashi S, Uchiumi T, Nuntagij A, Kotepong S, Suzuki A, Higashi S, Abe M (2004). A novel symbiotic nitrogen-fixing members of the *Ochrobactrum* clade isolated form root nodules of *Acacia mangium.* J. Gen. Appl. Microbiol. 50: 17-27.

Oliveres FL, James EK, Baldani JI, Dobereiner J (1997). Infection of mottled stripe disease susceptible and resistant sugarcane varieties by the endophytic diazotroph *Herbaspirillum*. New Phytol. 135: 723-737.

Perret X, Staehelin C, Broughton WJ (2000). Molecular basis of symbiotic promiscuity. Microbiol. Mol. Biol. Rev. 64: 180-201.

Polhill RM, Raven PH, Stirton CH (1981). Evolution and systematics of the Leguminosae. In: Polhill RM, P.H. Raven (eds) Advances in Legume Systematics Part 1, Royal Botanic Gardens, Kew, UK, pp. 1-26

Raja P, Uma S, Sundaram SP (2006). Non-nodulating pink-pigmented facultative *Methylobacterium* sp. with a functional *nifH* gene. World J. Microbiol. Biotechnol. 22: 1381-1384

Rasolomampianina R, Bailly X, Fetiarison R, Rabevohitra R, Bena G, Ramaroson L, Raherimandimy M, Moulin L, DeLajudie P, Dreyfus B (2005). Nitrogen fixing nodules from rose wood legume trees (*Dalbergia* spp) endemic to Madagascar host seven different genera belongs to α and β Proteobacteria. Mol. Ecol. 14: 4135-4146.

Rivas R, Velazquez E, Willems A, Vizcaino N, Subbarao NS, Mateos PF, Gillis M, Dazzo FB Molina EM (2002). A new species of *Devosia* that forms a unique nitrogen fixing root symbiosis with aquatic legume *Neptunia natans* (l.f) Druce. Appl. Environ. Microbiol. 68: 5217-5222.

Rivas R, Willems A, Subbarao NS, Mateos PF, Dazzo FB, Kroppenstedt RM, Martinez-Molina E, Gillis M, Velazquez E (2003). Description of *Devosia neptuniae* sp. nov. that nodulates and fixes nitrogen in symbiosis with *Neptunia natans,* an aquatic legume from India. Syst. Appl. Microbiol.26: 47-53.

Schultze M, Kondorosi A (1998). Regulation of symbiotic root nodule development. Annu. Rev. Genet. 32: 33-57.

Sullivan JT, Ronson CW (1998). Evolution of rhizobia by acquisition of a 500 kb symbiosis island that integrates into a phe-tRNA gene. Proc. Natl. Acad. Sci. USA 95: 5145-5149.

Sy A, Giraud E, Jourand P, Garcia N, Willems A, DeLajudie P, Prin Y, Neyra M, Gillis M, Boivin-Masson C Dreyfus B (2001). Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. J. Bacteriol. 183: 214-220.

Trujillo ME, Willems A, Abril A, Planchuelo AM, Rivas R, Ludena D, Mateos PF, Molina EM Velazquez E (2005). Nodulation of *Lupinus albus* by strains of *Ochrobactrum lupini* sp. Nov. Appl. Environ. Microbiol.71: 1318-1327.

Valverde A, Velazquez E, Gutierrez C, Cervantes E, Ventosa A, Igual JM (2003). *Herbaspirillum lusitanum* sp. nov., a novel nitrogen fixing bacterium associated with root nodules of *Phaseolus vulgaris.* Int. J. Syst. Evol. Microbiol. 53: 1979-1983.

Valverde A, Velazquez E, Santos FF, Vizcaino N, Rivas R, Mateos PF, Molina EM, Igual JM Willems A (2005). *Phyllobacterium trifolii* sp. nov., nodulating *Trifolium* and *Lupinus* in Spanish soils. Int. J. Syst. Evol. Microbiol. 55: 1985-1989.

Van Berkum P, Eardly BD (2002). The aquatic budding bacterium *Blastobacter denitrificans* is a nitrogen fixing symbiont of *Aeschynomene indica.* Appl. Environ. Microbiol. 68: 1132-1136.

Van Berkum P, Tully RE, Keister DL (1995). Non-pigmented and bacteriochlorophyll containing bradyrhizobia isolated from *Aeschynomene indica*. Appl. Environ. Microbiol. 1: 623-629.

Vandamme P, Coeyne T (2004). Taxonomy of the genus *Cupriavidus*: a tale of lost and found. Int. J. Syst. Evol. Microbiol. 54: 2285-2289.

Vandamme P, Goris J, Chen WM, de Vos P, Willems A (2002) *Burkholderia tuberum* sp. nov. and *Burkholderia phymatum* sp. nov. nodulate the roots of tropical legumes. Syst. Appl. Microbiol. 25: 507-512.

Young JPW, Haukka KE (1996). Diversity and phylogeny of Rhizobia. New Phytol. 133: 87-94.

Zakhia F, deLajudie P (2001).Taxonomy of rhizobia. Agronomie 21: 569-576.