Chapter 8
Legume Root Nodule Associated Bacteria

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Abstract Root nodules have intrigued mankind ever since their role in the maintenance of soil fertility has been known. The earlier school of thought amongst microbiologists and agronomists was that root nodules are highly specialised structures rich in leghaemoglobin, which house the diazotrophic bacterium *Rhizobium*, whose primary role was to fix atmospheric nitrogen in association with the host plant. But several path-breaking discoveries over the past few decades have thrown light on the plethora of bacterial occupants of the root nodules and their possible role in nodulation and N fixation besides several other beneficial roles. Recent technological advances in bacterial taxonomy and microbial ecology have unearthed a wide range of microbial nodule occupants, some of which have been encompassed under the classical umbrella of rhizobia, purely based on their ability to nodulate the host and fix atmospheric nitrogen, while other closely or even distantly related bacterial genera devoid of the ability to

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nodulate and fix nitrogen in nodules are often referred to as endophytes or simply nodule inhabitants. This chapter attempts to capture the existing knowledge on the root nodule associated bacteria both rhizobial and non-rhizobial and their possible roles in sustaining plant growth.

Introduction

The world over, legumes hold an important place in sustaining soil fertility and ensuring the nutritional security of both the human and animal populations. The uniqueness of this group of plants arises from the fact that they bear nodules, which serve as sites of nitrogen fixation, thereby enabling access of plants to ammoniacal nitrogen, a reduction product of atmospheric nitrogen. This process is mediated by the prokaryote – exclusive enzyme nitrogenase. Legumes are estimated to have evolved nearly 59 million years ago, with all three subfamilies recognisable soon after. Amongst the three subfamilies of legumes, nodulation is widespread in Papilionoideae, frequent in Mimosoideae and rare in Caesalpinioideae. This observation assumes significance since the subfamily Papilionoideae is thought to have been preceded by Mimosoideae and Caesalpinioideae (Allen and Allen 1981). Hence nodulating legumes are postulated to have evolved at a later time period in comparison to their non-nodulating relatives. An interesting question that arises at this point of time is that why nodulation evolved in some groups of legumes alone. Several lines of evidence suggest that, about 55 million years ago, when nodulated legumes evolved, there was a major peak in atmospheric carbon dioxide, temperature and humidity (Bowen et al. 2004), thereby creating an atmosphere of excess carbon dioxide. Since it is a well-established fact that the process of nitrogen fixation uses a significant amount of the carbon fixed by the host plant, a possible driving force behind the evolution of nodules could have been an excess of carbon dioxide coupled with deficiency of nitrogen. The first organisms that nodulated and colonised legume nodules presumably gained entry by the direct epidermal or crack infection. Subsequently, this led to two distinct modes of nodule development: one involving transcellular infection tubes, while the second mode was devoid of these specialised structures (Sprent 2007).

The recent past has noticed a surge of information on bacteria belonging to the α- and ß-proteobacterial groups, which are known to infect and nodulate legumes and have broadly accommodated under the umbrella term rhizobia. Apart from these, several bacterial genera and species exist in root nodules in the cryptic mode and are not known to harbour nodulation traits. Some of the early non-rhizobial bacteria isolated from legume nodules included *Agrobacterium* (De Lajudie et al. 1999) and *Bacillus* spp. (Bai et al. 2002). But later findings indicate that the nodule occupants can be as diverse as members of the genera *Inquilinus*, *Bosea*, *Rhodopseudomonas*, *Paracraurococcus*, *Phyllobacterium*, *Starkeya*, *Sphingomonas*, *Pseudomonas*, *Agromyces*, *Microbacterium*, *Ornithinicoccus* and *Paenibacillus*. Interestingly, most of these non-rhizobial bacterial genera are not known to play a role in nodule formation (Zakhia et al. 2006). The mode of entry of such bacteria into root nodules and their possible roles in plant metabolism still remain to be clearly deciphered. This chapter attempts to capture the existing knowledge on various legume root nodule associated bacteria and their possible roles in sustaining plant growth and soil fertility.

The Legume Root Nodule as an Ecological Niche for Bacteria: An Evolutionary Perspective

A unique feature of rhizobia that sets them apart from plant-associated bacteria is their ability to ultimately become intracellular symbionts within nodule cells. The two major papilionoid nodule groups, namely, the dalbergioid and genistoid legumes, appeared early, about 55 million year ago. The dalbergioid legumes are characterised by the presence of aeschynomenoid nodules that are devoid of uninfected cells in the infected region, and their infection processes do not involve transcellular infection tubes (Lavin et al. 2001). The genistoid legumes also share similar characteristics but have an indeterminate growth pattern unlike the dalbergioid nodules that have a determinate growth pattern. It has been postulated that the default position for infection in these legumes lies directly between the epidermal or cortical cells (Sprent and James 2007). It has been observed that as rhizobia pass between cells, they may be surrounded by some of the extra cellular components normally found in transcellular infection tubes. This mode of infection accounts for approximately 25 % of all legume genera (Brewin 2004).

The second mode of nodule development takes place in legumes, which are thought to have evolved later, probably in between 55 and 50 million years ago. This involves the transcellular infection tubes, although in some cases the tubes might not be necessarily involved in the infection process. The entry of transcellular infection tubes into newly formed meristematic cells is accompanied by cessation of later phases of mitotic division. This leads to polyploid cell development and the cells become enlarged, thereby enabling them to house vast numbers of nitrogen-fixing bacteria. Individual cells are infected by branches of the transcellular infection tubes, and the active nitrogen-fixing tissue contains a mixture of both infected and uninfected cells. This pattern of nodule development appears common in some members of Mimosoideae and all members of Caesalpinioideae (Sprent and James 2007). While the mode of entry, colonisation behaviour and the nodulation process of the rhizobial group of bacteria have been well established, the mode of entry of the non-rhizobial species and their colonisation behaviour still continue to intrigue microbiologists. It has been widely speculated that the non-rhizobial bacterial species either enter nodules through the cracks that appear at the time of lateral root emergence or some species may even hitch a ride along with rhizobia, while a later school of thought is that the non-rhizobial groups that exist in nodules are simply endophytes that exist within a nodule tissue without causing any external symptoms.

A Historical Perspective of Legume Root Nodule Associated Bacteria

In the beginning of the twentieth century, only one nodulating bacterium had been described, *Bacillus radicicola* (subsequently renamed as *Rhizobium*). This development was followed by the discovery of fast- and slow-growing rhizobia, which were subsequently given different generic names (*Rhizobium* and *Bradyrhizobium*). Subsequently, several genera of rhizobia infecting a wide variety of legumes and plant parts were recognised. The rhizobial genera initially associated with legume nodules were *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Sinorhizobium* (*Ensifer*), *Rhizobium* and *Mesorhizobium* (Zakhia and de Lajudie 2001). Currently, the International Committee on Systematics of Prokaryotes (ICSP), Subcommittee on Taxonomy of Rhizobium and Agrobacterium-Diversity, Phylogenetics and Taxonomy recognises 17 bacterial genera capable of nodulating and fixing atmospheric nitrogen in symbiosis with leguminous plants (http://edzna.ccg.unam.mx/rhizobial-taxonomy/node/4). These include 14 α-proteobacterial genera and three genera of β-proteobacteria. The latest genus to be included in this list is *Microvirga*, which is found to encompass three nodulating species in taxonomically separate legume hosts (Ardley et al. 2012).

A landmark discovery in rhizobial ecology was the discovery of the ability of *Burkholderia* and *Cupriavidus*, both belonging to the ß-class of proteobacteria to nodulate legumes (Chen et al. 2001; Moulin et al. 2001). This gains significance since it was believed that the nodulation trait was exclusively distributed amongst the α proteobacteria to which the classical *Rhizobium* and its related genera belong. A later development in β-proteobacteria was the inclusion of the genus *Herbaspirillum* as a nodulating bacterial species (Valverde et al. 2003). To encompass this massive development, the terms rhizobia/root-nodulating bacteria (RNB)/legume-nodulating bacteria (LNB) have been coined and have been used by various workers. But the underlying feature of all these terms is the ability of the bacterial species to nodulate and fix atmospheric nitrogen in association with various legume species. Some of the significant milestones in the discoveries of association between legumes and their root-nodulating bacteria are listed in Fig. 8.1.

Bacteria Associated with Legume Root Nodules

For the sake of brevity and better understanding, we have classified the root nodule-associated bacteria into two sections, namely, rhizobial and non-rhizobial, with emphasis on the non-rhizobial bacteria that are associated with nodules and their functional role in plant growth and development.

- 1679-Malphigi describes “lumps” on legume roots
- 1829-Meyen makes observations on legume root associated bacteria
- 1866-Woronin confirms Meyen’s Hypotheses
- 1888-Hermann Hellriegel and Hermann Wilfarth discover the nitrogen contribution potential of nodules
- 1888-Martinus Beijerinck isolated the nodule associated bacteria by enrichment technique and names them *Bacillus radicicola*
- 1889-Renamed as *Rhizobium leguminosarum* by Frank
- 1932-Fred proposes the cross inoculation grouping of Rhizobia
- 1942-Cohn discovers *Agrobacterium*
- 1964-Distinguishing of fast and slow growing rhizobia by Graham
- 1982-Discovery of *Bradyrhizobium*
- 1988-Discovery of *Azonhizobium* and *Sinorhizobium*
- 1997-Discovery of *Mesorhizobium* and presence of several non rhizobial bacteria in root nodules
- 1998-Discovery of *Allorhizobium*
- 2001-Discovery of root nodulation by β-proteobacteria
- 2004-Claim of nodulation by γ proteobacteria
- 2012-*Microvirga* included as a root nodulating bacteria

Fig. 8.1 Significant milestones in legume root associated bacterial discovery

Rhizobial Occupants of Legume Root Nodules

The evolution of rhizobial taxonomy from a single species to the present-day umbrella of rhizobia has been a long winding path, where several candidate genus and species were included/excluded over extended periods of time, while several original genera/species and some others have not been proved conclusively. Currently, the ICSP Subcommittee on Taxonomy of Rhizobium and Agrobacterium-Diversity, Phylogenetics and Taxonomy recognises 17 bacterial genera, namely, *Allorhizobium*, *Aminobacter*, *Azorhizobium*, *Bradyrhizobium*, *Devosia*, *Ensifer*, *Mesorhizobium*, *Methylobacterium*, *Microvirga*, *Ochrobactrum*, *Phyllobacterium*, *Rhizobium*, *Shinella*, *Sinorhizobium* (*Ensifer*), *Burkholderia*, *Cupriavidus* and *Herbaspirillum*, which are capable of nodulating and fixing atmospheric nitrogen in symbiosis with leguminous plants (http://edzna.ccg.unam.mx/rhizobial-taxonomy/node/4). The last three genera that are listed above constitute the beta-proteobacterial group within the rhizobial framework (Fig. 8.2).

The genus *Burkholderia* comprises of the following nodulating species, namely, *Burkholderia tuberum* (Vandamme et al. 2002), *B. phymatum* (Vandamme et al. 2002), *B. mimosarum* (Chen et al. 2006), *B. nodosa* (Chen et al. 2007), *B. sabiae* (Chen et al. 2008), *B. caribensis* (Chen et al. 2003), *B. contaminans* (Vanlaere et al. 2009), *B. fungorum* (Coenye et al. 2001), *B. lata* (Vanlaere et al. 2009) and *B. symbiotica* (Sheu et al. 2012). The symbiosis-related genes of *Burkholderia* are thought to have diverged over a long period within *Burkholderia* without substantial horizontal gene transfer between species complexes (Bontemps et al. 2010). An interesting feature of rhizobial taxonomy is that often the same genus or even species contains both rhizobial and non-rhizobial strains; for example, the genus *Methylobacterium* contains one rhizobial species *M. nodulans* (Jourand et al. 2004), in addition to several saprophytic species. Similarly, *Cupriavidus* (formerly *Ralstonia taiwanensis*) species is known to have been isolated from nodules as well as clinical samples (Chen et al. 2001). Therefore, it would be ideal to assess the nodulation potential of a bacterial strain and detect the presence of *nod* and *nif* genes, before assigning it to the

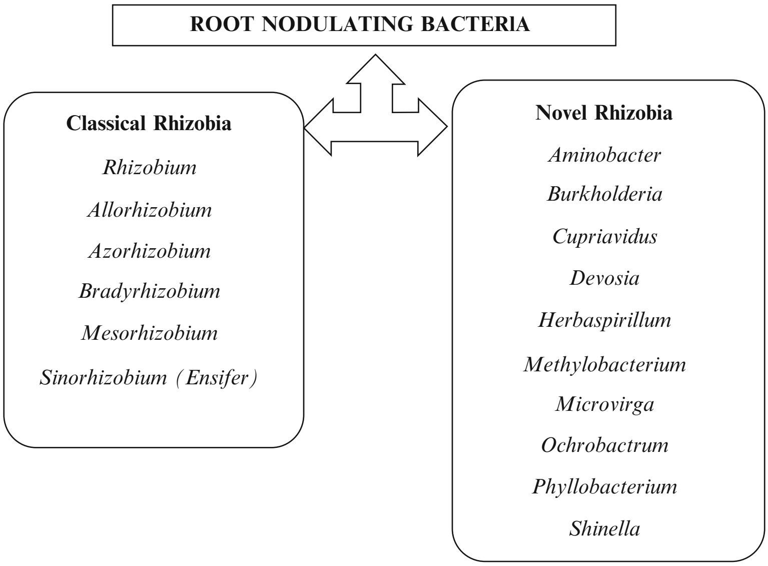

Fig. 8.2 Classical and novel rhizobial genera that are known to nodulate legumes

broad umbrella of rhizobia. Table 8.1 presents a list of the nonclassical rhizobia that have been known to be associated with various leguminous plants.

Though each rhizobial species has a specific host spectrum, there is no strict correlation between legume and bacterium taxonomy, and very often the same bacterium has been recovered from more than one host. However, some associations are known to be favoured in nature (e.g. *Azorhizobium–Sesbania* and *Burkholderia–Mimosa*).

Novel Rhizobia and Diazotrophy

Till date nodulation and diazotrophy remain the buzz words in rhizobial explorations, and hence, the world has witnessed an explosion in the number of nodulating and N-fixing bacterial species. With the explosion in knowledge on novel rhizobial, one would naturally expect the utilisation of these bacterial strains in inoculum development for crop production. But, unfortunately, the converse is true and till date the most favoured rhizobial strains used for crop production are limited species of the classical *Rhizobium* and *Bradyrhizobium* and to a limited extent *Sinorhizobium* (*Ensifer*). If one were to explore the reasons for this, it is evident that most novel nodulating bacteria have been discovered from wild legumes and their nitrogen-fixing abilities have been attributed solely to the presence of the *nod* and *nif H* gene. With the exception of a handful of experiments, very few studies

Table 8.1 Published nonclassical rhizobia associated with various legumes and their features

Bacterial species	Host	Feature	Reference
Blastobacter denitrificans	*Aeschynomene indica*	Presence of *nif* HDK gene	Van Berkum and Eardly (2002)
Devosia neptuniae	*Neptunia natans*	*nod* D gene of *Devosia* is closely related to *R. leguminosarum*	Rivas et al. (2002)
Devosia yakushimensis	*Pueraria lobata*	Isolated from the nodules, renodulation not reported	Bautista et al. (2010)
Ensifer adhaerens	*Sesbania grandiflora*, *Medicago sativa*, *etc*.	Isolated from multiple genera	Merabet et al. (2010), Willems et al. (2003)
Ensifer arboris	*Acacia senegal*, *Prosopis chilensis*	Isolated from multiple genera	Nick et al. (1999)
Ensifer fredii	*Glycine* spp., *Vigna unguiculata* and *Cajanus cajan*	Isolated from multiple genera	Chen et al. (1988)
Ensifer kostiense	*Acacia senegal*, *Prosopis chilensis*	Isolated from multiple genera	Nick et al. (1999)
Ensifer kummerowiae	*Kummerowiae stipulacea*	Single host	Wei et al. (2002)
Ensifer medicae	*Medicago* spp.	Isolated from multiple hosts	Rome et al. (1996)
Ensifer meliloti	*Medicago* spp., *Melilotus* spp.	Isolated from multiple genera	Rome et al. (1996)
Ensifer mexicanus	*Acacia* spp., *Phaseolus vulgaris*	Isolated from multiple genera	Lloret et al. (2007)
Ensifer morelense	*Leucaena leucocephala*	Single host	Wang et al. (2002)
Ensifer numidicus	*Argyrolobium uniflorum*	Single host	Merabet et al. (2010)
Ensifer saheli	*Sesbania* spp.	Single host	De Lajudie et al. (1994)
Ensifer teranga	*Sesbania* spp.	Isolated from multiple hosts	Lortet et al. (1996)
Ensifer xinjiangense	*Glycine max*	Single host	Chen et al. (1988)
Methylobacterium nodulans	*Crotalaria* spp.	*M. nodulans* contains *nod* ABC gene and genes encoding structural nitrogenase enzyme	Sy et al. (2001)
Ochrobactrum lupini	*Lupinus albus*	The nodulating and nitrogen-fixing genes (*nod* and *nif* genes) were detected in all the *sym* plasmids using *nif H* and *nod D* probes	Trujillo et al. (2005)
Ochrobactrum cytisi	*Cytisus scoparius*	Single host	Zurdo-Pineiro et al. (2007)

(continued)

Table 8.1 (continued)

Bacterial species	Host	Feature	Reference
Ochrobactrum cytisi	*Lupinus albus*, *Lupinus honoratus*	Single host	Trujillo et al. (2005)
Phyllobacterium trifolii	*Trifolium pratense*, *Trifolium repens* and *Lupinus albus*	It harbours symbiotic plasmids which have nodulating and nitrogen-fixing genes	Valverde et al. (2005)
Phyllobacterium ifriqiyense	*Trifolium pratense*, *T. repens* and *Lupinus albus*	Isolated from multiple genera	Mantelin et al. (2006)
Phyllobacterium leguminum	*Astragalus algerianus* and *Argyrolobium uniflorum*	Isolated from multiple genera	Mantelin et al. (2006)
Shinella kummerowiae	*Kummerowia stipulacea*	Unable to renodulate the original host plant	Lin et al. (2008)
Burkholderia caribensis	*Mimosa pudica*, *M. diplotricha*	Single host	Chen et al. (2003), Vandamme et al. (2002)
Burkholderia dolosa	*Alysicarpus glumaceus*	Only one strain isolated from the host plant	Vandamme et al. (2002)
Burkholderia mimosarum	*Mimosa pigra*, *M. scabrella*	Single host	Chen et al. (2006)
Burkholderia nodosa	*Mimosa bimucronata*, *M. scabrella*	Single host	Chen et al. (2007)
Burkholderia phymatum	*Machaerium lunatum*	Presence of *nod* ABC shows the capability to produce nod factors to initiate nodulation	Vandamme et al. (2002)
Burkholderia tuberum	*Aspalathus carnosa*	Presence of *nod* ABC shows the capability to produce nod factors to initiate nodulation	Vandamme et al. (2002)
Burkholderia sabiae	*Mimosa caesalpineafolia*	Single host	Chen et al. (2008)
Cupriavidus taiwanensis	*Mimosa* spp.	Single host	Chen et al. (2003)
Cupriavidus necator	*Mimosa caesalpineafolia* , *L. leucocephala*, *Macroptilium atropurpureum*, *P. vulgaris* and *Vigna unguiculata*	Isolated from multiple genera	da Silva et al. (2012)
Herbaspirillum lusitanum	*Phaseolus vulgaris*	Single host	Valverde et al. (2003)

Note: The nomenclature of some published species has not been validated by the ICSP Subcommittee on the Taxonomy of *Rhizobium* and *Agrobacterium* have been carried out with the novel rhizobial in order to establish their diazotrophic potential. In one of the available studies, Garu et al. (2009) studied the symbiotic capabilities of the beta-proteobacteria *Burkholderia phymatum* STM815^T and *Cupriavidus taiwanensis* LMG 19424^T, when inoculated onto the papilionoid legumes *Rhynchosia ferulifolia*, *R. caribaea*, *Rhynchosia minima* and *Macroptilium atropurpureum* (Siratro). The root nodule bacteria isolated from *R. minima* and *R. totta* were also included in the study. The level of N fixation by this symbiosis was reported to be almost as efficient as that of the *Medicago* symbiosis. While the molecular evidence and taxonomic validation of such novel strains are definitely of interest, the utility of such rhizobia in terms of N contribution to the host and the soil on which it grows has been poorly established. This requires the determination of the ability of the novel rhizobia to nodulate a wide spectrum of cultivated legumes, besides studies such as the classical acetylene reduction assay (ARA) and ^{15}N studies. But the unfortunate part is that these crucial studies have not received the attention of microbiologists and agronomists the word over, and hence to this day the realm of rhizobial inoculant usage has not moved beyond the boundaries of a handful of species. The ^{15}N isotope dilution technique (Talbott et al. 1982) continues to be a preferred method for determination of the N-fixing potential of any legume–rhizobia symbiosis. Hence, much more information needs to be generated by this technique using the novel rhizobial and a wide range of host legumes.

Non-rhizobial Occupants of Legume Root Nodules

The later part of the last century was dotted with findings that lead to a surge in the explorations of various legume root nodules and exposed a plethora of bacteria that were hitherto known to exist in association with legume nodules. The observations that legume root nodules play hosts to diverse microbes like *Bacillus*, *Streptomyces*, *Herbaspirillum*, Arbuscular Mycorrhizal Fungi and *Agrobacterium* (Sturz et al. 1997; De Lajudie et al. 1999; Tokala et al. 2002; Valverde et al. 2003; Scheublin et al. 2004) gave rise to a school of thought that they were probably endophytes. But the term 'endophyte' has been much debated, and for a bacterial species to be denoted as a 'true endophyte' more stringent evaluation than mere isolation from surface-sterilised plant tissue is suggested (Schulz and Boyle 2006). Hence, most non-rhizobial bacteria found in root nodules are commonly referred to as nodule inhabitants. It would not be far-fetched to say that many of these initial observations probably led to our present-day understanding of the novel nodulating bacterial genera that fall outside the classical *Rhizobium*.

Sturz et al. (1997) made a novel observation that the legume root nodule is known to accommodate several eubacterial genera apart from rhizobia and their population densities are reported to be in the range of 10^4 viable bacteria per gram of fresh nodule tissue. A pioneering observation made by them was that clover root nodules were host to 12 bacteria species other than rhizobia, including eight tissue-specific ones. Interestingly, it was reported that *R. leguminosarum* bv. *trifolii* constituted only 8.8 % of all the root nodule bacteria recovered. In another early report, Bai et al. (2002) reported the isolation of putative endophytic *Bacillus* including a growth-promoting *Bacillus thuringiensis* strain from the nodules of soybean plants. This was followed by a dramatic claim that nodules of the legume *Hedysarum* were nodulated by bacteria belonging to the class Gammaproteobacteria (Benhizia et al. 2004). This claim was based on the lack of any rhizobial-like sequence on amplification of the bulk of microbial cells obtained from the squashed nodules. The authors therefore speculated that the exclusive occupants of the nodules formed by the three plants belonged to the orders Enterobacteriales or Pseudomonadales. The bacterial species implicated in the nodulation process include *Pantoea agglomerans*, *Enterobacter kobei*, *Enterobacter cloacae*, *Leclercia adecarboxylata*, *Escherichia vulneris* and *Pseudomonas* sp. But till date, this finding is yet to gain credibility amongst rhizobial workers, and the status of the bacterial species remains more of endophytes rather than true nodulants.

Later Wang et al. (2006a) detected the presence of *Pantoea*, *Erwinia*, *Salmonella*, *Enterobacter*, *Citrobacter* and *Klebsiella* in nodules of the tree species *Conzattia multiflora* grown in Mexico. The presence of *Agrobacterium* strains in nodules, but incapable of nodulating their hosts, has been frequently reported from the nodules of different legumes, and various possible mechanisms have been proposed to explain the existence of these bacteria within nodule tissue (De Lajudie et al. 1999; Han et al. 2005). Wang et al. (2006b) proved that the *Agrobacterium* strain CCBAU 81181, which was originally isolated from the root nodules of *Onobrychis viciifolia*, and a symbiotic strain of *Sinorhizobium meliloti* CCBAU 10062 could actually co-inhabit the root nodules of *Melilotus dentatus*. Kan et al. (2007) concluded from a study of 61 root nodule isolates from diverse legumes, namely, *Vicia*, *Oxytropis*, *Medicago*, *Melilotus* and *Onobrychis* species grown in Qinghai–Tibet plateau, that in addition to nodulating genera like *Rhizobium leguminosarum*, *S. meliloti*, *Sinorhizobium fredii*, *Mesorhizobium* sp. and *Phyllobacterium* sp., two non-symbiotic groups related to *Agrobacterium* and Enterobacteriaceae were present in their nodules. Selvakumar et al. (2008) reported the presence of diverse plant growth promoting strains of bacteria such as *Bacillus thuringiensis*, *Enterobacter asburiae* and *Serratia marcescens* from the nodules of the legume *Kudzu* (*Pueraria thunbergiana*) grown in the Indian Himalayan Region. Dashti et al. (2009) made an unusual finding that the surfaces of root nodules of *Vicia faba* and *Lupinus albus* were colonised by bacterial consortia that utilised oil and fixed nitrogen. This finding has immense value in the realm of nitrogen-poor desert soils where anthropogenic oil spills are quite common. The nodules of peanut grown in Argentina were found to harbour Gammaproteobacteria predominantly belonging to the genera *Pseudomonas* spp., *Enterobacter* spp. and *Klebsiella* spp. These strains enhanced plant yield and colonised preformed nodules when co-inoculated with an effective bradyrhizobial strain (Ibánẽz et al. 2009). The presence of endophytic bacteria belonging to Alphaproteobacteria, Betaproteobacteria, Actinobacteria and Firmicutes phyla encompassing nine different

Table 8.2 Some non-rhizobial bacteria associated with legume root nodules and their features

Bacterial species	Host plant	Features	Reference(s)
Agrobacterium-like strains	*Phaseolus vulgaris*, *Acacia*, *Prosophis*, *Chamaecrista*	Nitrogen-fixing genes were detected	Mhamdi et al. (2002), De Lajudie et al. (1999)
Labrys neptuniae	*Neptunia oleracea*	Novel species	Chou et al. (2007)
Microbacterium sp. and *Starkeya* sp.	Spontaneous legumes	Presence of *nif* H-like gene detected	Zakhia et al. (2006)
Bacillus megaterium, *Brevibacillus choshinensis* and *Microbacterium trichothecenolyticum*	*Medicago sativa*	Plant growth promotion traits	Stajković et al. (2009)
Bacterial isolates with maximum similarity to *Bacillus subtilis*, *Bacillus simplex* and *Agrobacterium tumefaciens*	*Vigna radiata*	IAA production, P solubilisation	Tariq et al. (2012)

genera, namely, *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Dyella*, *Methylobacterium*, *Microbacterium*, *Rhizobium* and *Staphylococcus*, from the nodules of the legume *Lespedeza* sp. grown in two different locations in South Korea was reported by Palaniappan et al. (2010). Most of the isolates they studied showed multiple plant growth promotion activity, i.e. indole acetic acid production, ACC deaminase activity, siderophore production and phosphate solubilisation.

The knowledge about the plethora of bacterial nodule inhabitants has expanded and some interesting reports have started to emerge. The existence of plant-borne lineages of *Salmonella* was an interesting observation, with public health implications (Wang et al. 2006a). Muresu et al. (2010) observed that nodules of three wild legumes of the genus *Hedysarum* grown in Algeria harboured potential human pathogenic bacterial strains such as *Enterobacter cloacae*, *Enterobacter kobei*, *Escherichia vulneris*, *Pantoea agglomerans* and *Leclercia adecarboxylata*. These strains exhibited pathogenic traits such as cytotoxicity, vital strain exclusion and adhesion to epithelia. In a recent report, the presence of coccobacilli was reported from the root nodules of fenugreek (*Trigonella foenum-graecum*). An interesting observation here is that 64.7 % of the bacterial occupants were Gram-negative coccobacilli and 29.41 % were Gram-positive bacilli. Two isolates possessing maximum positive PGP features belonged to the genus *Exiguobacterium* (Rajendran et al. 2012), which is probably the first for this genus. The existence of *Micromonospora* in nodules of *Lupinus angustifolius* collected from Spain has been recently reported by Trujillo et al. (2010). Table 8.2 lists some of the non-rhizobial bacteria that are known to occur in legume root nodules.

Functional Role of Legume Root Nodule Associated Bacteria

While the primary role of rhizobia is the conversion of atmospheric nitrogen to ammonia, through an energy-intensive reduction process, it has been variably argued that the micro-symbiont drains the host of its energy resources (Vance and Heichel 1991). But this school of thought has been debated since the overall benefit the plant and the surrounding ecosystem derive as a result of the nitrogen fixation process far outweighs the drain on the carbon resources of the plant. In contrast to the rhizobia whose role is largely confined to diazotrophy, non-rhizobial nodule occupants seem to have a diverse influence on the plant survival, nodulation and growth promotion and yield (Remans et al. 2008; Selvakumar et al. 2008). It has been hypothesised that IAA of bacterial origin from the nodules is transported to other plant parts (Basu and Ghosh 1998) and dually occupied nodules serve as hot spots for lateral gene transfer of symbiotic genes from rhizobia (Valverde et al. 2005). In general, the non-rhizobial bacteria are thought to synergistically act with rhizobia and increase nodulation and yield possibly by production of growth hormones like IAA production, solubilisation of nutrients, N fixation and siderophore production. In view of this, researchers tend to focus their attention towards the isolation and characterisation of non-rhizobial bacteria from legume nodule and utilising these strains to improve nodulation and crop growth yields.

The symbiotic effectiveness of rhizobia can be improved by co-inoculation with suitable non-rhizobial beneficial bacteria in most legume crops (Lazdunski et al. 2004). In some studies, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter* and *Kurthia* have also been evaluated with rhizobia and were found to improve plant growth (Pandey and Maheshwari 2007). Hung et al. (2007) isolated endophytic bacteria from surface-sterilised stems, roots and nodules of wild and cultivated soybean varieties. They analysed various phenotypic traits that are expected to be involved in the persistence and functions of these bacteria. Most of the isolates from soybean were motile, indole acetic acid producers capable of cellulase and pectinase activities. A strain of *Bacillus thuringiensis* originally isolated from nodules of the wild legume *Kudzu* was able to promote plant growth and nodulation of soybean when inoculated with *Bradyrhizobium japonicum* (Mishra et al. 2008). The same Bt strain when co-inoculated with *R. leguminosarum* improved plant growth and nodulation of pea and lentil (Mishra et al. 2009).

The role of nodule bacteria in the selection of the rhizobial stains was revealed by Mrabet et al. (2006) who observed in soils of Tunisia, that nodulation of common beans showed a biased genetic structure, with high levels of inhibition of *Rhizobium gallicum*, while nodulation by *Sinorhizobium medicae* was favoured. The co-inoculation of non-sterile soils with *R. gallicum* and *Agrobacterium* confirmed these findings. In vitro antibiosis assays indicated that agrobacteria possessed a significant antagonism against *R. gallicum*. The positive effect of co-inoculation of non-rhizobial endophytes isolated from sterilised root nodules of alfalfa (*Medicago sativa* L.) and *Sinorhizobium meliloti*, on nodule numbers in alfalfa, as compared to

S. meliloti inoculation alone has been reported by Stajković et al. (2009). The existence of 99 % similarities in the *nif* H genes of *Bradyrhizobium japonicum* and the endophytic *Bacillus* strains strongly indicates the possibility of horizontal transfer of symbiotic genes between the symbiotic bacteria and the endophytes (Li et al. 2008). The nodulation behaviour of soybean seems to have an effect on the endophytic occupants of soybean stems. A greater abundance of Firmicutes was observed in Nod^{-} (non-nodulating) and Nod^{++} (hyper-nodulating mutants) of soybeans, compared to the wild type (Okubo et al. 2009). A few interpretations that can be drawn from the available information are that non-rhizobia occur in significant numbers and influence the rhizobial microbial composition of the nodule. Most of these isolates posses plant growth promotion traits. But their definite role within the legume root nodule needs to be established.

Conclusion

Interest in legume nodule microbiology has grown by leaps and bounds over the years, and with the continuing addition to the existing knowledge on nodule-occupying bacteria and their functional role, it is being increasingly recognised that the nodule harbours not only symbiotic 'rhizobia' but also a wide plethora of non-rhizobial organisms that play both well-established and cryptic roles in plant metabolism. The frontiers of science are being pushed beyond their boundaries, which is much evident from the numbers of nodulating and nitrogen-fixing genera, which have been broadly accommodated under the umbrella term 'rhizobia'. But most of these developments remain as artefacts of academic interest, and their utility in terms of inoculant production and utilisation has remained largely unexplored. Similarly, the ever-expanding knowledge on non-rhizobial nodule-associated bacterial species has also remained confined to the pages of academic journals, while their practical utility has not seen the dawn of the day. Some future lines of research could be:

1. Determination of the cross inoculation potential of the novel rhizobia in association with cultivated legumes
2. Establishment of the N-fixing potential of the novel rhizobia, by classical methods such as the ARA assay and ^{15}N dilution studies
3. Quantification of the diazotrophic benefits of the novel rhizobial–legume association by isotopic and non-isotopic methods
4. Exploration and utilisation of the non-rhizobia as inoculants in association with rhizobia, preferably in the consortia mode in order to promote effective nodulation.

Therefore, it needs to be emphasised that the utility of both rhizobial and non-rhizobial–legume nodule associated bacteria will be realised fully only when they move beyond the confinement of the publication space and tend to be utilised as inoculants in order to harness their potential for the nutritional security of mankind.

References

Allen ON, Allen EK (1981) The *Leguminosae*: a source book of characteristics, uses and nodulation. University of Wisconsin Press/Macmillan Publishing, Madison/London

Ardley JK, Parker MA, De Meyer SE, Trengove RD, O'Hara GW, Reeve WG, Yates RY, Dilworth MJ, Willems A, Howieson JG (2012) *Microvirga lupini* sp. nov., *Microvirga lotononidis* sp. nov., and *Microvirga zambiensis* sp. nov. are alphaproteobacterial root nodule bacteria that specifically nodulate and fix nitrogen with geographically and taxonomically separate legume hosts. Int J Syst Evol Microbiol 62:2579–2588

Bai YM, Daoust F, Smith DL, Driscoll BT (2002) Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. Can J Microbiol 48:230–238

Basu PS, Ghosh AC (1998) Indole acetic acid and its metabolism in root nodules of a monocotyledonous tree *Roystonea regia*. Curr Microbiol 37:137–140

Bautista VV, Monsalud RG, Yokota A (2010) *Devosia yakushimensis* sp. nov., isolated from root nodules of *Pueraria lobata* (Willd.) Ohwi. Int J Syst Evol Microbiol 60:627–632

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(4):462–468

Bontemps C, Elliot GN, Simon MF, Fabio B, Gross E, Lawton RC, Neto NE, Da Fa M, De FaLouriero TM, De Farion SM, Spernt JI, James EK, Young PW (2010) *Burkholderia* species are ancient symbionts of legumes. Mol Ecol 19:44–52

Bowen GJ, Beerling DJ, Koch PL, Zachos JC, Quattlebaum T (2004) A humid climate state during the Palaeocene/Eocene thermal maximum. Nature 432:495–499

Brewin NJ (2004) Plant cell wall remodelling in the *Rhizobium*-legume symbiosis. CRC Crit Rev Plant Sci 23:293–326

Chen WX, Yan GH, Li LJ (1988) Numerical taxonomic study of fast-growing soybean rhizobial and proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int J Syst Bacteriol 38:392–397

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, Boivin-Masson C (2003) Legume symbiotic nitrogen fixation by β proteobacteria is widespread in nature. J Bacteriol 185:7266–7272

Chen WM, James EK, Coenye T, Chou JH, Edmundo B, de Faria SM, Elliott GN, Sheu SY, Sprent JI, Peter V (2006) *Burkholderia mimosarum* sp. nov., isolated from root nodules of *Mimosa* spp. from Taiwan and South America. Int J Syst Evol Microbiol 56:1847–1851

Chen WM, de Faria SM, James EK, Elliott GN, Lin KY, Chou JH, Sheu SY, Cnockaert M, Sprent JI, Vandamme P (2007) *Burkholderia nodosa* sp. nov., isolated from root nodules of the woody Brazilian legumes *Mimosa bimucronata* and *Mimosa scabrella*. Int J Syst Evol Microbiol 57:1055–1059

Chen WM, de Faria SM, Chou JH, James EK, Elliott GN, Sprent JI, Bontemps C, Young JPW, Vandamme P (2008) *Burkholderia sabiae* sp. nov., isolated from root nodules of *Mimosa caesalpiniifolia*. Int J Syst Evol Microbiol 58:2174–2179

Chou YJ, Elliott GN, James EK, Lin KY, Chou JH, Sheu SY, Sheu DS, Sprent JI, Chen WM (2007) *Labrys neptuniae* sp. nov., isolated from root nodules of the aquatic legume *Neptunia oleracea*. Int J Syst Evol Microbiol 57:577–581

Coenye T, Laevens S, Willems A, Ohlen M, Hannant W, Govan JRW, Gillis M, Falsen E, Vandamme P (2001) *Burkholderia fungorum* sp. nov. and *Burkholderia caledonica* sp. nov., two new species isolated from the environment, animals and human clinical samples. Int J Syst Evol Microbiol 51:1099–1107

da Silva K, Florentino LA, da Silva KB, de Brandt E, Vandammec V, de Souza Moreira FM (2012) *Cupriavidus necator* isolates are able to fix nitrogen in symbiosis with different legume species. Syst Appl Microbiol 35:175–182

Dashti N, Khanafer M, El-Nemr IN, Sorkhoh N, Radwan AS (2009) The potential of oil-utilizing bacterial consortia associated with legume root nodules for cleaning oily soils. Chemosphere 74:1354–1359

De Lajudie P, Willems A, Pot B, Dewettinck D, Maestrojuan G, Neyra M, Collins MD, Dreyfus B, Kersters K, Gillis M (1994) Polyphasic taxonomy of rhizobia: emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. nov., *Sinorhizobium saheli* sp. nov., and *Sinorhizobium teranga* sp. nov. Int J Syst Bacteriol 44:715–733

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

Garu G, Yates RJ, Deiana P, Howieson JG (2009) Novel strains of nodulating *Burkholderia* have a role in nitrogen fixation with papilionoid herbaceous legumes adapted to acid, infertile soils. Soil Biol Biochem 41:125–134

Han SZ, Wang ET, Chen WX (2005) Diverse bacteria isolated from root nodules of *Phaseolus vulgaris* and species within the genera *Campylotropis* and *Cassia* grown in China. Syst Appl Microbiol 28:265–276

Hung PQ, Kumar SM, Govindsamy V, Annapurna K (2007) Isolation and characterization of endophytic bacteria from wild and cultivated soybean varieties. Biol Fertil Soils 44:155–162

Ibánẽz F, Angelini J, María TT, Tonelli L, Fabra A (2009) Endophytic occupation of peanut root nodules by opportunistic Gammaproteobacteria. Syst Appl Microbiol 32:49–55

Jourand P, Giraud E, Bena G, Sy A, Willems A, Gillis M, Dreyfus B, de Lajudie 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

Kan FL, Chen ZY, Wang ET, Tian CF, Sui XH, Chen WX (2007) Characterization of symbiotic and endophytic bacteria isolated from root nodules of herbaceous legumes grown in Qinghai–Tibet plateau and in other zones of China. Arch Microbiol 188:103–115

Lavin M, Pennington RT, Klitgaard BB, Sprent JI, de Lima HC, Gasson PE (2001) The Dalbergioid legume (*Fabaceae*): delimitation of a pantropical monophyletic clade. Am J Bot 88:503–533

Lazdunski AM, Ventre I, Sturgis JN (2004) Regulatory circuits and communication in Gram-negative bacteria. Nat Rev Microbiol 2:581–592

Li JH, Wang ET, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol Biochem 40:238–246

Lin DX, Wang ET, Tang H, Han TX, He YR, Guan SH, Chen WX (2008) *Shinella kummerowiae* sp. nov., a symbiotic bacterium isolated from root nodules of the herbal legume *Kummerowia stipulacea*. Int J Syst Evol Microbiol 58:1409–1413

Lloret L, Ormeño-Orrillo E, Rincón R, Martinez-Romero J, Rogel-Hernandez MA, Martinez-Romero E (2007) *Ensifer mexicanus* sp. nov. a new species nodulating *Acacia angustissima* (Mill.) Kuntze in Mexico. Syst Appl Microbiol 30:280–290

Lortet G, Mear N, Lorquin J, Dreyfus B, de Lajudie P, Rosenberg C, Boivin C (1996) Nod factor thin-layer chromatography profiling as a tool to characterize symbiotic specificity of rhizobial strains: application to *Sinorhizobium saheli*, *S. teranga* and *Rhizobium* sp. strains isolated from *Acacia* and *Sesbania*. Mol Plant-Microbe Interact 9:736–747

Mantelin S, Fischer-Le Saux M, Zakhia F, BenaG BS, Jeder H, de Lajudie P, Cleyet-Marel J-C (2006) Emended description of the genus *Phyllobacterium* and description of four novel species associated with plant roots: *Phyllobacterium bourgognense* sp. nov., *Phyllobacterium ifriqiyense* sp. nov., *Phyllobacterium leguminum* sp. nov. and *Phyllobacterium brassicacearum* sp. nov. Int J Syst Evol Microbiol 56:827–839

Merabet C, Martens M, Mahdhi M, Zakhia F, Sy A, Coopman R, Bekki A, Mars M, Willems A, de Lajudie P (2010) Multilocus sequence analysis of root nodule isolates from *Lotus arabicus* (Senegal), *Lotus creticus*, *Argyrolobium uniflorum* and *Medicago sativa* (Tunisia) and description of *Ensifer numidicus* sp. nov. and *Ensifer garamanticus* sp. nov. Int J Syst Evol Microbiol 60:664–674

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

Mishra PK, Mishra S, Selvakumar G, Kundu S, Gupta HS (2008) Enhanced soybean (*Glycine max* L.) plant growth and nodulation by *Bradyrhizobium japonicum* SB1 in presence of *Bacillus thuringiensis*-KR1. Acta Agric Scand Sect B-Plant Soil Sci 59:189–196

Mishra PK, Mishra S, Selvakumar G, Kundu S, Bisht JK, Gupta HS (2009) Coinoculation of *Bacillus thuringeinsis*-KR1 with enhances plant growth and nodulation of Pea (*Pisum sativum* L.) and Lentil (*Lens culinaris* L.). World J Microbiol Biotechnol 25:753–761

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

Mrabet M, Mnasri B, Romdhane SB, Íe Laguerre G, Aouani ME, Mhamdi R (2006) *Agrobacterium* strains isolated from root nodules of common bean specifically reduce nodulation by *Rhizobium gallicum*. FEMS Microbiol Ecol 56:304–309

Muresu R, Maddau G, Delogu G, Cappuccinelli P, Squartini A (2010) Bacteria colonizing root nodules of wild legumes exhibit virulence-associated properties of mammalian pathogens. Antonie van Leeuwenhoek 97:143–153

Nick G, de Lajudie P, Eardly BD, Suomalainen S, Paulin L, Zhang X, Gillis M, Lindström K (1999) *Sinorhizobium arboris* sp. nov. and *Sinorhizobium kostiense* sp. nov., isolated from leguminous trees in Sudan and Kenya. Int J Syst Bacteriol 49:1359–1368

Okubo T, Ikeda S, Kaneko T, Eda S, Mitsuyi H, Sato S, Tabata S, Minamisawa K (2009) Nodulation-dependent communities of culturable soybean endophytes from stems of field grown endophytes. Microb Environ 24:253–258

Palaniappan P, Chauhan PS, Saravanan VS, Anandham R, Sa T (2010) Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. Biol Fertil Soils 46:807–816

Pandey P, Maheshwari DK (2007) Two-species microbial consortium for growth promotion of *Cajanus cajan*. Curr Sci 92:1137–1142

Rajendran G, Patel MH, Joshi SJ (2012) Isolation and characterization of nodule-associated *Exiguobacterium* sp. from the root nodules of Fenugreek (*Trigonella foenum-graecum*) and their possible role in plant growth promotion. Int J Microbiol. doi: 10.1155/2012/693982

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Gutierrez RT, El-Howeity M, Michiels J, Vanderleyden J (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). Plant Soil 302:149–161

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

Rome S, Fernandez MP, Brunel B, Normand P, Cleyet-Marel JC (1996) *Sinorhizobium medicae* sp. nov., isolated from annual *Medicago* spp. Int J Syst Bacteriol 46:972–980

Scheublin TR, Ridgway KP, Young JP, van der Heijden MG (2004) Non legumes, legumes and root nodules harbour different arbuscular mycorrhizal fungal communities. Appl Environ Microbiol 70:6240–6246

Schulz B, Boyle C (2006) What are endophytes. In: Schulz B, Boyle C, Sieber TN (eds) Microbial root endophytes, vol 9. Springer, Berlin

Selvakumar G, Kundu S, Gupta AD, Shouche YS, Gupta HS (2008) Isolation and characterization of nonrhizobial plant growth promoting bacteria from nodules of *Kudzu* (*Pueraria thunbergiana*) and their effect on wheat seedling growth. Curr Microbiol 56:134–139

Sheu SY, Chou JH, Bontemps C, Elliott GN, Gross E, James EK, Sprent JI, Young PW, Chen WM (2012) *Burkholderia symbiotica* sp. nov., isolated from root nodules of *Mimosa* spp. native to north-east Brazil. Int J Syst Evol Microbiol 62:2272–2278

Sprent JI (2007) Evolving ideas of legume evolution and diversity: a taxonomic perspective of the occurrence of nodulation. New Phytol 171:11–25

Sprent JI, James EK (2007) Legume evolution: where do nodules and mycorrhizas fit in? Plant Physiol 144:575–581

Stajković O, De Meyer S, Miličić B, Willems A, Delić D (2009) Isolation and characterization of endophytic non-rhizobial bacteria from root nodules of alfalfa (*Medicago sativa* L.). Bot Serbica 33:107–114

Sturz AV, Christie BR, Matheson BG, Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fertil Soils 25:13–19

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

Talbott HJ, Kenworthy WJ, Legg JO (1982) Field comparison of the ^{15}N and difference methods of measuring nitrogen fixation. Agron J 74:799–804

Tariq M, Hameed S, Yasmeen T, Amanat A (2012) Non-rhizobial bacteria for improved nodulation and grain yield of mung bean [*Vigna radiata* (L.) Wilczek]. Afr J Biotechnol 11:15012–15019

Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra MJ (2002) Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl Environ Microbiol 68:2161–2171

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 lupine* sp. nov. Appl Environ Microbiol 71:1318–1327

Trujillo ME, Alonso-Vega P, Rodríguez R, Carro L, Cerda E, Alonso P, Martínez-Molina E (2010) The genus *Micromonospora* is widespread in legume root nodules: the example of *Lupinus angustifolius*. ISME J 4:1265–1281

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, Fernandez-Santos F, 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

Vance CP, Heichel GH (1991) Carbon in N_2 fixation: limitation or exquisite application. Annu Rev Plant Physiol Plant Mol Biol 42:373–390

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

Vanlaere E, Baldwin A, Gevers D, Henry D, De Brandt E, LiPuma JJ, Mahenthiralingam E, Speert DP, Dowson D, Vandamme P (2009) Taxon K, a complex within the *Burkholderia cepacia* complex, comprises at least two novel species, *Burkholderia contaminans* sp. nov. and *Burkholderia lata* sp. nov. Int J Syst Evol Microbiol 59:102–111

Wang ET, Tan ZY, Willems A, Fernández-López M, Reinhold-Hurek B, Martínez-Romero E (2002) *Sinorhizobium morelense* sp. nov., a *Leucaena leucocephala* associated bacterium that is highly resistant to multiple antibiotics. Int J Syst Evol Microbiol 52(5):1687–1693

Wang ET, Tan ZY, Guo XW, Rodríguez-Duran R, Boll G, Martínez-Romero E (2006a) Diverse endophytic bacteria isolated from a leguminous tree *Conzattia multixora* grown in Mexico. Arch Microbiol 186:251–259

Wang LL, Wang ET, Liu J, Li Y, Chen WX (2006b) Endophytic occupation of root nodules and roots of *Melilotus dentatus* by *Agrobacterium tumefaciens*. Microb Ecol 52:436–443

Wei GH, Wang ET, Tan ZY, Zhu ME, Chen WX (2002) *Rhizobium indigoferae* sp. nov. and *Sinorhizobium kummerowiae* sp. nov., respectively isolated from *Indigofera* spp. and *Kummerowia stipulacea*. Int J Syst Evol Microbiol 52:2231–2239

Willems A, Fernández-López M, Muñoz E, Goris J, Martínez-Romero E, Toro N, Gillis M (2003) Description of new *Ensifer* strains from nodules and proposal to transfer *Ensifer adhaerens*

Cassida 1982 to *Sinorhizobium* as *Sinorhizobium adhaerens* comb. nov. Request for an Opinion. Int J Syst Evol Microbiol 53:1207–1217

Zakhia F, de Lajudie P (2001) Taxonomy of rhizobia. Agronomie 21:569–576

Zakhia F, Jeder H, Willems A, Gillis M, Dreyfus B, de Lajudie P (2006) Diverse bacteria associated with root nodules of spontaneous legumes in Tunisia and first report for *nif* H like gene within the genera *Microbacterium* and *Starkeya*. Microb Ecol 51:375–393

Zurdo-Piñeiro JL, Rivas R, Trujillo ME, Vizcaíno N, Carrasco JA, Chamber M, Palomares A, Mateos PF, Martínez-Molina E, Velázquez E (2007) *Ochrobactrum cytisi* sp. nov., isolated from nodules of *Cytisus scoparius* in Spain. Int J Syst Evol Microbiol 57:784–788