Chapter 7
Diversity of Interactions Between Rhizobia and Legumes

Abstract The relationship between rhizobia and legumes can be specific or promiscuous. Some rhizobia can nodulate a diverse range of legumes, while other rhizobia only infect specific legumes. From the plant perspective, some legumes can be nodulated only by specific rhizobia, while other legumes can be nodulated by different genera of rhizobia. Various interactions between rhizobia and legumes are reviewed in this chapter. In addition, different infection patterns, nodule morphology and development and types of bacteroids in nodule cells are briefly discussed.

7.1 Rhizobia with Broad Host Ranges

Every rhizobium has its own host range. Some rhizobia like *Sinorhizobium meliloti* can nodulate with only specific host legume, *Melilotus* and *Medicago*, and these rhizobia are classified as narrow host-range rhizobia. In contrast, some rhizobia can infect and nodulate many different kinds of legumes, and they are described as broad or wide host-range rhizobia.

Strain NGR234 (NGR is the abbreviation for New Guinea *Rhizobium*) was the only fast-growing strain among 30 isolates prepared from *Lablab purpureus* nodules grown in Papua New Guinea (soil pH 8.5) by Trinick in 1965 (Pueppke and Broughton 1999). The bacterium NGR234 nodulates a very wide range of hosts, with more than 112 genera of legumes (Pueppke and Broughton 1999) as well as the nonlegumes *Parasponia andersonii* (Op den Camp et al. 2012), *Parasponia rugosa* and *Parasponia parviflora* in the family Cannabaceae (Behm et al. 2014). The hosts infected by strain NGR234 are evolutionarily divergent legumes with fundamental differences in nodule morphology and physiology. Strain NGR234-infected nodules of different legumes include both determinate and indeterminate nodules. The common legumes forming determinate nodules infected by NGR234 are represented by *Lablab purpureus* (Li et al. 2013), *Vigna unguiculata* (Li et al. 2013; Pueppke and Broughton 1999) and *Lotus japonicus* (Pueppke and Broughton 1999), while indeterminate nodules formed by NGR234 are exemplified by its well-studied interactions with *Leucaena leucocephala* (Li et al. 2013) and *Sophora flavescens* (Jiao et al. 2015b).

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However, the broad host-range strain NGR234 cannot nodulate some widely used and studied legumes such as *Medicago sativa*, *Medicago truncatula*, *Pisum sativum*, *Vicia faba*, *Cicer arietinum*, *Astragalus sinicus*, *Arachis hypogaea*, *Glycine max* (only some varieties could be nodulated by NGR234 and the nodules formed are inefficient), *Trifolium repens* and *Phaseolus vulgaris*. The inability to nodulate these important model legumes restrict its use in some extensive and deep studies, but NGR234 has nevertheless been used in some important studies of the symbiotic relationship between rhizobia and legumes (Li et al. 2013; Müller et al. 2001; Xin et al. 2012; Xu et al. 2018; Zamani et al. 2017).

NGR234 is a *Sinorhizobium*, closely related to *S. fredii* but perhaps representing a distinct species (Tian et al. 2012). Besides strain NGR234, other rhizobial strains have also been found to have wide host ranges. Examples are various strains of *Sinorhizobium fredii*: HH103, USDA257 and CCBAU 45436. Strain HH103 can nodulate *Lotus burttii*, *Glycine max* (Acosta-Jurado et al. 2016), *Glycyrrhiza uralensis* (Crespo-Rivas et al. 2016) and *P. vulgaris* (Sadowsky et al. 1988). Strain USDA257 can form nodules with 79 legume plant genera (Pueppke and Broughton 1999; Schuldes et al. 2012). Strain CCBAU 45436 can establish effective symbiosis with *G. max* (determinate nodules) and *Cajanus cajan* (indeterminate nodules) (Li et al. 2016).

7.2 Rhizobia Associated with Symbiotically Specific Plants

7.2.1 Mesorhizobia and Chickpea

Belonging to the tribe Cicereae, chickpea (*Cicer arietinum*) is, after soybean, the second most widely grown legume crop. It is grown in nearly 50 countries around the world (Varshney et al. 2013), particularly in India, Australia and arid and semiarid countries around the Mediterranean Basin. In relation to its symbiotic nitrogen fixation (SNF) with rhizobia and its ability to grow on depleted soils with low rainfalls, chickpea cultivation presents both huge agricultural significance and environmental security, decreasing fertiliser input, promoting the growth of chickpea itself and the crops rotated with it and providing human dietary protein, especially in developing counties. Through SNF, chickpea can supply nitrogen to the soil ranging from 80 to 141 kg ha^{-1} when inoculated with an efficient rhizobial strain (Herridge et al. 1995). Rhizobial population richness, genetic diversity, geographic distribution, symbiotic matching, soil properties and inoculation with an effective strain can influence the effectiveness of SNF between the two symbiotic partners.

Chickpea rhizobia were first assigned to the genus *Rhizobium* by Nour et al. with two species described, *Rhizobium ciceri* in 1994 (Nour et al. 1994) and *Rhizobium mediterraneum* in 1995 (Nour et al. 1995). However, it became apparent that these species were sufficiently diverged to require the creation of a new genus, and because the intermediate growth speed of chickpea rhizobia is slower than that of fast growers like *Rhizobium* species but faster than the slow growers like *Bradyrhizobium* species, a novel genus *Mesorhizobium* (meso-, middle, intermediate) was proposed, and these two species were subsequently transferred to *Mesorhizobium* gen. nov. (Jarvis et al. 1997), with *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* as their new combined names in 1997. In 2012, a third novel species *Mesorhizobium muleiense* was proposed by Zhang et al. (2012a) when they studied the distinct chickpea rhizobia in Xinjiang China (Zhang et al. 2012b). An additional species was isolated from root nodules of chickpea grown in Gansu Province of Northwest China (Zhang et al. 2017) and has now been named *Mesorhizobium wenxiniae* (Zhang et al. 2018). There are reports of other potential novel *Mesorhizobium* species nodulating chickpea in southern and central Ethiopia (Tena et al. 2017) and in Indian soils (Nandwani and Dudeja 2009).

Besides the above four species, chickpea can be nodulated by many other *Mesorhizobium* that were originally described from hosts other than chickpea, including *M. amorphae*, *M. tianshanense* and *M. opportunistum* isolated from chickpea nodules in Spain and Portugal (Laranjo et al. 2012; Rivas et al. 2007) and *M. tarimense*, *M. gobiense*, *M. tamadayense*, *M. opportunistum*, *M. loti* and *M. amorphae* isolated from chickpea nodules in eastern Algeria (Dekkiche et al. 2017).

Endophytic bacteria, including *Sinorhizobium* (*Ensifer*) spp., were also isolated from chickpea nodules in some arid regions. In a survey of chickpea rhizobia in Algeria, 41 isolates were assigned to *Ensifer meliloti*, while 19 isolates could firmly be considered as *Mesorhizobium* strains (Dekkiche et al. 2017). Of the 15 randomly chosen *Ensifer* isolates, only 2 could form root nodules and were verified to be identical to the original isolated strain (Dekkiche et al. 2017). However, *nodC* gene could not be amplified from any of the 41 *E. meliloti* isolates, though 9 isolates had a *nifH* gene closely related to the *nifH* gene in *E. meliloti* and *E. medicae* (Dekkiche et al. 2017). Other studies also reported the existence of *Ensifer* spp. in chickpea nodules (Aouani et al. 2001; Ben Romdhane et al. 2007). Some other bacteria, including *Agrobacterium* sp., *Ochrobactrum* sp. (Naseem et al. 2005) and *Rhizobium* (Ogutcu et al. 2009), have also been isolated from chickpea nodules. The efficiency of nitrogen fixation of these endophytic non-*Mesorhizobium* isolates on chickpea needs to be checked. The role of these apparently non-symbiotic endophytic bacteria is unclear, but they might be helping in the process of nodulation (Hameed et al. 2004) or have the function of increasing phosphate solubilisation (Hameed et al. 2005).

From studies on the phylogeny of symbiosis genes (*nodC* and *nifH*) of chickpea rhizobia, it is clear that all these different chickpea-nodulating *Mesorhizobium* spp. have very similar symbiosis genes and these differ from the genes of rhizobia associated with non-chickpea legumes (Fig. 7.1). This could explain the highly host specificity of chickpea (Dadarwal 1980), and all these chickpea-nodulating rhizobia should be assigned to the symbiovar *ciceri*, i.e. they are *Mesorhizobium* spp. sv. *ciceri*. The distinct symbiosis genes are found in different *Mesorhizobium* spp. in different countries, indicating strong positive selection by chickpea. However, specific chickpea *Mesorhizobium* species were found only in specific regions. Retention and horizontal transfer of the chickpea-specific symbiosis genes among different mesorhizobia may be common to keep the specific symbiosis with chickpea.

Fig. 7.1 Phylogenetic tree based on *nodC* genes of different sv. *ciceri* strains nodulating *Cicer arietinum* and *Cicer canariense*. *Sinorhizobium medicae* A-321 was used as outgroup. Strains from different continents (Europe, Asia, Africa) were highlighted with different colours. Bar, 5% nucleotide substitution

So far, only *M. muleiense* and *M. wenxiniae* have been found in nodules of chickpea in China. *M. ciceri* is, however, found to be widely distributed in the Mediterranean, North Africa, North America, Indian subcontinent and Russia (Nour et al. 1995). The wide distribution of *M. ciceri* may be due to its adaption to soils with a wide range of pH from 5.0 to 10.0 and its temperature resistance up to 40 °C (Jarvis et al. 1997). *M. muleiense* from chickpea nodules has only been found in China, especially in alkaline soils (pH 8.46–9.16) in Xinjiang Province, where chickpea has been cultivated for over 2500 years (Zhang et al. 2012a, b, 2014). Ten strains isolated from nodules of *Astragalus* in Ningxia and Shanxi Provinces were classified as *M. muleiense*, but their symbiosis genes (*nodC* and *nifH*) were different from those of sv. *ciceri* (Yan et al. 2016a). The complete genome sequence of *M. ciceri* sv. *biserrulae* strain WSM1271 (isolated from root nodules of the pasture legume *Biserrula pelecinus* introduced into Australian soil in 1994) (Nandasena et al. 2014) and the ongoing genome sequencing (unpublished) of four sv. *ciceri* strains belonging to different species (*M. ciceri*, *M. mediterraneum*, *M. muleiense*, *M. wenxiniae*) will help scientists to elucidate the distinct origin of their symbiosis genes and their transfer among different *Mesorhizobium* species.

Cicer canariense is a perennial wild chickpea endemic to the Canary Islands, and nine *Mesorhizobium* genospecies, including *M. ciceri*, were found to nodulate this wild plant (Armas-Capote et al. 2014). The other eight species included *M. caraganae*, *M. opportunistum*, *M. tamadayense*, *M. australicum*, *M. loti* and three (*M. tianshanense*/*M. gobiense*/*M. metallidurans*) that could not be unambiguously assigned based on 16S rRNA gene phylogeny (Armas-Capote et al. 2014). Only a small group of isolates had *nodC* sequences identical to those of sv. *ciceri*, and these were all *M. ciceri* (Armas-Capote et al. 2014). The other isolates have two distinct *nodC* lineages, one close to *Lotus* symbionts such as *M. loti* NZP2213^{T} and the other differing from all known mesorhizobia (Armas-Capote et al. 2014). Besides these *Mesorhizobium* spp., some fast-growing strains belonging to *Rhizobium leguminosarum* whose symbiotic *nodC* gene was not close to bv. *ciceri* but close to *R. leguminosarum* bv. *trifolii* ATCC 14480 phylogenetically have been reported to nodulate *C. canariense* effectively (Martinez-Hidalgo et al. 2015). A LacZ-labelled derivative of strain *R. leguminosarum* bv. *trifolii* RCCHU01 was used to confirm the effective nodulation on *C. canariense* and *Trifolium repens* (Martinez-Hidalgo et al. 2015). These results indicate that the domesticated chickpea (*C. arietinum*) is more selective in its choice of rhizobia than the wild chickpea (*C. canariense*).

7.2.2 **Sinorhizobium/Ensifer** *and Alfalfa*

It is considered that the *Sinorhizobium* (*Ensifer*) *meliloti* is the most effective and widely distributed rhizobial species in interacting with alfalfa (*Medicago sativa* L.) in symbiotic nitrogen-fixing (Wang et al. 2018), though the other minor species of *S. medicae* (Rome et al. 1996), *Rhizobium tibeticum* (Hou et al. 2009) and *R. favelukesii* (Torres Tejerizo et al. 2016) were reported to nodulate alfalfa.

Results from the analyses of 581 nodule isolates of alfalfa grown in Tibetan Plateau have been shown, 579 of them were classified as *S. meliloti*, only 1 isolate was identified as *S. medicae*, and 1 isolate was identified as *Rhizobium* sp. (close to *R. mongolense* and *R. yanglingense* based on *nodC* phylogeny) (Wang et al. 2018), supporting the extremely wide distribution and preponderance of *S. meliloti* in Tibetan Plateau. Similarly, the dominant species from alfalfa nodules in Serbian soil was also *S. meliloti*, and only one strain was identified as *S. medicae*, two strains as *R. tibeticum* and one strain as *Rhizobium* sp. (Stajković-Srbinović et al. 2012). This is also true in Argentina and Uruguay (Segundo et al. 1999).

In summary, alfalfa is a highly selective and specific legume that prefers *S. meliloti* as its effective symbiont around the world.

7.2.3 **Mesorhizobium amorphae** *and* **Amorpha fruticosa**

Amorpha fruticosa is a shrub legume indigenous to North America (Allen and Allen 1981) that was introduced to China as a windbreak and as soil cover for erosion control. The majority of rhizobia forming an effective symbiosis with *A. fruticosa* growing in different regions of China were characterised as a sole species of *Mesorhizobium*, *M. amorphae* (Wang et al. 1999). The type strain of *M. amorphae*, ACCC 19665^T, only nodulates its host plant *A. fruticosa* in cross-nodulation tests (Wang et al. 1999). In 2002, Wang et al. (2002) reported that *A. fruticosa* in its native North America was also nodulated by *M. amorphae*, which was probably transmitted from the USA to China.

In 2007, Gu et al. identified several rhizobial strains isolated from *Lespedeza* species as *M. amorphae* (Gu et al. 2007), and two strains (CCBAU 45224 and CCBAU 25056) had *nodC* and *nifH* genes completely identical those of type strain ACCC 19665^T. Cross-nodulation tests among the *M. amorphae* populations isolated, respectively, from *A. fruticosa* and *Lespedeza* spp. would be helpful to reveal if they can nodulate both the host plants.

7.3 Rhizobia Associated with Promiscuous Plants

7.3.1 Various Rhizobia and Soybean (**Glycine max**)

Two genera, the *Bradyrhizobium* and *Sinorhizobium* (syn. *Ensifer*), are the publicly recognised rhizobia that can establish effective symbiosis with soybean (*Glycine max*). Two isolates from nodules of soybean grown in Xinjiang were previously classified as *Rhizobium* (now *Mesorhizobium*) *tianshanense* (Chen et al. 1995), but their authentication could not be verified later (our unpublished observations). The current subsection only focuses on the two well-known genera of soybean rhizobia.

In the genus *Bradyrhizobium*, seven rhizobial species including *B. elkanii* (Kuykendall et al. 1992), *B. japonicum* (Jordan 1982), *B. diazoefficiens* (Delamuta et al. 2013), *B. daqingense* (Wang et al. 2013a), *B. liaoningense* (Xu et al. 1995), *B. huanghuaihaiense* (Zhang et al. 2012c) and *B. ottawaense* (Yu et al. 2014) have been described until now to nodulate soybean. In the phylogenetic tree based on six concatenated housekeeping gene sequences (Fig. 7.2), these seven soybean bradyrhizobial species occupied their distinct positions. However, only two *nodC* gene types were found among these seven species, with *B. elkanii* as a sole type; the other six species had identical *nodC* gene sequences (see Chap. 5).

In the genus *Sinorhizobium*, four fast-growing species of soybean symbionts have been established: *S.* (*E.*) *fredii* (de Lajudie et al. 1994; Jarvis et al. 1992), *E. sojae* (Li et al. 2011), *E. glycinis* (Yan et al. 2016b) and *E. shofinae* (Chen et al. 2017). Based on the maximum likelihood phylogenetic tree of three housekeeping genes (Chen et al. 2017), the four fast-growing soybean rhizobia occupy distinct positions among the other known species in *Ensifer/Sinorhizobium* (Fig. 7.3).

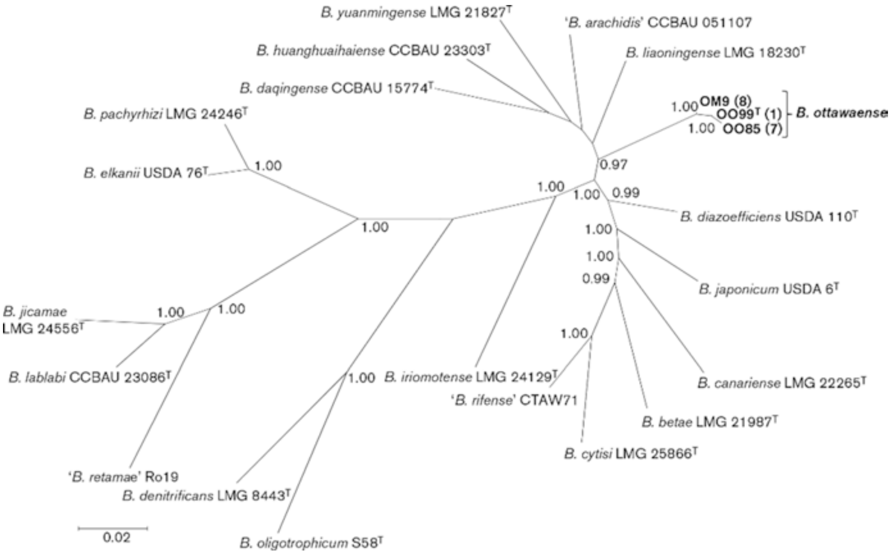

Fig. 7.2 Bayesian phylogenetic tree of *atpD-glnII-recA-gyrB-rpoB-dnaK* concatenated gene sequences for species in genus *Bradyrhizobium* (Yu et al. 2014)

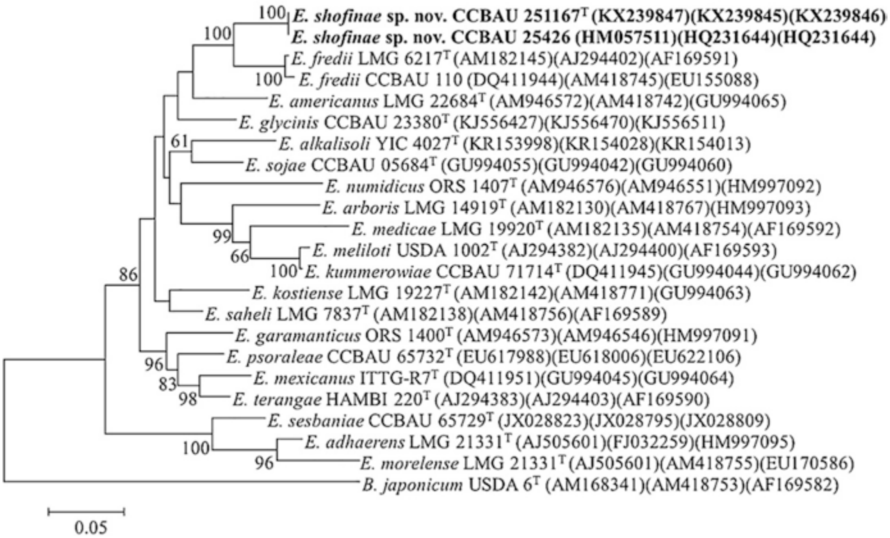

Fig. 7.3 Maximum likelihood (ML) phylogenetic tree based on concatenated sequences of *recA*, *atpD* and *glnII* showing the genetic relationship among type strains of species in genus *Ensifer* (*Sinorhizobium*) (Reprint from Chen et al. (2017))

As with the *nodC* gene in *Bradyrhizobium* species, all the four *Ensifer* species had identical *nodC* genes, too (see Chap. 5). In addition, the common components and structure of Nod factors (LCOs) secreted by these soybean-associating *Bradyrhizobium* and *Sinorhizobium/Ensifer* species may endow them with same host range (see Chap. 5).

7.3.2 Various Rhizobia and Sophora

Sophora is a leguminous genus in the tribe Sophoreae, the subfamily Faboideae. This genus contains about 50 species (Sprent 2009) of shrubs and small trees and is distributed worldwide. About 19 species in the genus *Sophora* have been documented to have nitrogen-fixing nodules (Sprent 2009), and for some species, their rhizobial diversity and taxonomy have been studied in detail since 1995 when Chen et al. described a novel species, *Rhizobium tianshanense* (Chen et al. 1995) (now renamed *Mesorhizobium tianshanense* (Jarvis et al. 1997)), from *Sophora alopecuroides* growing in Xinjiang, China. Another two rhizobial species, *Bradyrhizobium elkanii* (Han et al. 2008a) and *Rhizobium multihospitium* (Han et al. 2008b) were isolated from *S. alopecuroides* by Han et al. Five rhizobial strains isolated from other *Sophora* species growing in New Zealand were defined as groups related to two *Mesorhizobium* species and one *Rhizobium* species (Weir et al. 2004). Seventy-five nodule isolates of *S. alopecuroides* grown in China's Loess Plateau were clustered into nine genospecies in five genera, *Mesorhizobium*, *Agrobacterium*, *Phyllobacterium*, *Rhizobium* and *Sinorhizobium* (Zhao et al. 2010), revealing the promiscuous symbiosis of this legume. Cross-nodulation tests further confirmed that not only alpha-rhizobia (α-rhizobia) but also beta-rhizobia (β-rhizobia) could nodulate *Sophora flavescens* (Jiao et al. 2015a), another extremely promiscuous host plant, sister of *S. alopecuroides*. The extreme diversity of *nodC* types of various rhizobia that nodulate *S. flavescens* has been confirmed (Jiao et al. 2015a; Liu et al. 2018a).

The rhizobial species isolated from nodules of *Sophora* species are listed in Table 7.1, and they are classified into five genera as mentioned above. Altogether, 15 rhizobial (geno)species were clustered into the genus *Mesorhizobium*, isolated from 6 *Sophora* species. Fourteen rhizobial (geno)species were classified into the genus *Rhizobium*; five species were found in *Sinorhizobium/Ensifer*; two species in each of *Phyllobacterium* and *Bradyrhizobium*; and only one species was in the genus *Agrobacterium* (Table 7.1). Clearly, the predominant rhizobia of *Sophora* belong to the genera *Mesorhizobium* and *Rhizobium*.

The phylogeny of *nodC* and *nodA* symbiosis genes of the *Sophora*-associated rhizobia revealed diverse branches (Jiao et al. 2015a; Zhao et al. 2010). Five clades (13 types) of *nodA* genes were found in different *Sophora* rhizobia by Zhao et al. (Jiao et al. 2015a). Seven types of *nodC* genes were detected among different *Sophora* rhizobia in the study of Jiao et al. (Jiao et al. 2015a). Clearly, *Sophora* can be nodulated by rhizobia with phylogenetically different symbiosis genes. Interestingly, all the 51 *Mesorhizobium* strains belonging to 7 different species col-

Table 7.1 Various rhizobia isolated from root nodule of *Sophora* species

Rhizobial genus/species	*Sophora* spp.	Geographic origin	References
***Mesorhizobium* (15 spp.)**			
M. tianshanense	*S. alopecuroides*	Xinjiang, China	Chen et al. (1995) and Jarvis et al. (1997)
M. alhagi	*S. alopecuroides*	Gansu, Ningxia and Shaanxi, China	Zhao et al. (2010)
M. gobiense	*S. alopecuroides*	Gansu and Ningxia China	Zhao et al. (2010)
M. amorphae	*S. alopecuroides*	Ningxia, China	Zhao et al. (2010)
M. calcicola	*S. longicarinata*; *S. flavescens*	Marlborough, New Zealand Shaanxi, China	De Meyer et al. (2016) and Jiao et al. (2015a)
M. waitakense	*S. microphylla*	Otago, New Zealand	De Meyer et al. (2016)
M. sophorae	*S. microphylla*	Westland, New Zealand; Shaanxi, China	De Meyer et al. (2016) and Jiao et al. (2015a)
M. newzealandense	*S. prostrata*	Marlborough, New Zealand	De Meyer et al. (2016)
M. kowhaii	*S. microphylla*	Canterbury, New Zealand	De Meyer et al. (2016)
M. waimense	*S. longicarinata*	Waima/Ure River, Marlborough, New Zealand	De Meyer et al. (2015)
M. cantuariense	*S. microphylla*	Canterbury, New Zealand	De Meyer et al. (2015)
M. ciceri	*S. tetraptera*	New Zealand	Weir et al. (2004)
M. huakuii	*S. flavescens*	Shaanxi, China	Jiao et al. (2015a)
M. septentrionale	*S. flavescens*	Liaoning, Shanxi, China	Jiao et al. (2015a)
M. sp. ICMP 14330	*S. microphylla*	New Zealand	Weir et al. (2004)
***Rhizobium* (14 spp.)**			
R. indigoferae	*S. alopecuroides*	Ningxia and Gansu, China	Zhao et al. (2010)
R. giardinii	*S. alopecuroides*	Ningxia, China	Zhao et al. (2010)
R. leguminosarum	*S. chathamica*	New Zealand	Weir et al. (2004)
R. multihospitium	*S. alopecuroides*	Xinjiang, China	Han et al. (2008b)
R. sophorae	*S. flavescens*	Shanxi, China	Jiao et al. (2015c)
R. sophoriradicis	*S. flavescens*	Shanxi, China	Jiao et al. (2015c)
R. sp. II CCBAU 03429	*S. flavescens*	Shanxi, China	Jiao et al. (2015a)
R. sp. I CCBAU 03360	*S. flavescens*	Shanxi, China	Jiao et al. (2015a)
R. sp. III CCBAU 03419	*S. flavescens*	Shanxi, China	Jiao et al. (2015a)
R. sp. V CCBAU 11560	*S. flavescens*	Liaoning, China	Jiao et al. (2015a)
R. lusitanum	*S. flavescens*	Shanxi, China	Jiao et al. (2015a)
R. yanglingense	*S. flavescens*	Liaoning, China	Jiao et al. (2015a)
R. mongolense	*S. flavescens*	Liaoning, China	Jiao et al. (2015a)
R. pusense	*S. tomentosa*	Rio de Janeiro, Brazil	Toma et al. (2017)

(continued)

Table 7.1 (continued)

Rhizobial genus/ species	*Sophora* spp.	Geographic origin	References
***Sinorhizobium* (5 spp.)**			
S. fredii	*S. alopecuroides*; *S. flavescens*	Ningxia, Shanxi, China	Jiao et al. (2015a) and Zhao et al. (2010)
S. meliloti	*S. alopecuroides*	Gansu, China	Zhao et al. (2010)
S. (*E.*) *adhaerens*	*S. tomentosa*	Rio de Janeiro, Brazil; Taiwan, China	Hung et al. (2005) and Toma et al. (2017)
S. mexicanus	*S. tomentosa*	Rio de Janeiro, Brazil	Toma et al. (2017)
S. chiapanecum	*S. tomentosa*	Rio de Janeiro, Brazil	Toma et al. (2017)
***Agrobacterium* (1 spp.)**			
A. tumefaciens	*S. alopecuroides*	Gansu, China	Zhao et al. (2010)
***Phyllobacterium* (2 spp.)**			
P. trifolii	*S. alopecuroides*	Gansu and Ningxia, China	Zhao et al. (2010)
P. sophorae	*S. flavescens*	Shanxi, China	Jiao et al. (2015d)
***Bradyrhizobium* (2 spp.)**			
B. elkanii	*S. alopecuroides*; *S. flavescens*	Xinjiang, Shaanxi, Gansu, China	Han et al. (2008a) and Jiao et al. (2015a)
B. sp. I CCBAU 03416	*S. flavescens*	Shanxi, China	Jiao et al. (2015a)

lected from New Zealand had almost identical *nodC* genes, and they clustered closely with the mesorhizobia collected from China (Jiao et al. 2015a; Nguyen et al. 2017), indicating their common origin. Furthermore, identical *nodC* genes were found in isolates of two different genera, *R. mongolense* CCBAU 11559 and *M. septentrionale* CCBAU 11523, supporting the lateral gene transfer of nodulation genes among different rhizobial genera (Jiao et al. 2015a).

To test the promiscuous nodulation of *Sophora*, more rhizobial species belonging to different genera with different *nodC* phylogeny in the classes *Alphaproteobacteria* and *Betaproteobacteria* were chosen and cross-inoculated on *Sophora.* This identified another 26 rhizobial species that could nodulate *S. flavescens* (Table 7.2) (Jiao et al. 2015a). Consistent with the observed diversity of rhizobial species and symbiosis genes among isolates from *S. flavescens*, the cross-nodulation tests further confirmed the promiscuity of this plant, in that not only does it not select strictly the rhizobial species, but it does not select strictly the symbiosis gene types and accepts rhizobia originally isolated from different cross-nodulation groups. For example, *S. meliloti* and *M. huakuii* are two very host-specific rhizobial species, only selecting *Medicago* and *Astragalus sinicus*, respectively, as their host plant, but both of them can nodulate *S. flavescens* (Jiao et al. 2015a).

Although *S. flavescens* is nodulated promiscuously by many different rhizobia, there are still some rhizobial strains that cannot nodulate it, including the following: *Bradyrhizobium japonicum* USDA 6, *B. diazoefficiens* USDA 110, *B. yuanmingense* CCBAU 10071, *Bradyrhizobium* sp. strain ORS278, *B. oligotrophicum* LMG 10732, *Ochrobactrum lupini* LMG 22726, *M. muleiense* CCBAU 83963, *M. amor-*

Table 7.2 Cross-nodulation on *Sophora flavescens* by different rhizobia

Rhizobia	Original host	Nodulation
Alphaproteobacteria		
Bradyrhizobium elkanii USDA76	*Glycine max*	Yes
B. arachidis CCBAU 051107	*Arachis hypogenea*	Yes
Rhizobium yanglingense CCBAU 71623	*Gueldenstaedtia multiflora*	Yes
R. yanglingense CCBAU 01603	*Caragana intermedia*	Yes
R. fabae CCBAU 33202	*Vicia faba*	Yes
R. leguminosarum sv. *viciae* USDA 2370	*Pisum sativum*	Yes
R. tropici CIAT 899	*Phaseolus vulgaris*	Yes
R. pisi DSM 30132	*Pisum sativum*	Yes
R. laguerreae FB206	*Vicia faba*	Yes
R. vallis CCBAU 65647	*Phaseolus vulgaris*	Yes
R. etli bv. *phaseoli* CFN42	*Phaseolus vulgaris*	Yes
R. leguminosarum sv. *trifolii* LMG 8820	*Trifolium pratense*	Yes
Sinorhizobium meliloti USDA 1002	*Medicago sativa*	Yes
S. meliloti 1021	*Medicago sativa*	Yes
S. sp. NGR234	*Lablab purpureus*	Yes
S. fredii CCBAU 45436	*Glycine max*	Yes
Mesorhizobium loti NZP 2213	*Lotus corniculatus*	Yes
M. huakuii 7653R	*Astragalus sinicus*	Yes
M. qingshengii CCBAU 33460	*Astragalus sinicus*	Yes
M. tianshanense USDA 3592	*Glycyrrhiza pallidiflora*	Yes
M. temperatum CCBAU 01578	*Caragana intermedia*	Yes
M. amorphae CCBAU 01583	*Caragana intermedia*	Yes
M. septentrionale CCBAU 03074	*Astragalus membranaceus*	Yes
Phyllobacterium sophorae CCBAU 03422	*Sophora flavescens*	Yes
Betaproteobacteria		
Burkholderia tuberum LMG 21444	*Aspalathus carnosa*	Yes
Bur. sp. ICMP 19869	*Dipogon lignosus*	Yes
Bur. dipogonis ICMP 19430	*Diphgon lignosus*	Yes
Cupriavidus taiwanensis LMG 19424	*Mimosa pudica*	Yes

phae ATCC 19665, *R. galegae* HAMBI 1174, *Burkholderia nodosa* LMG 23741 and *Burkholderia phymatum* STM 815 (Jiao et al. 2015a). One of these specific rhizobia, USDA 110, a strain isolated from soybean (*Glycine max*), was studied further to find the molecular mechanism determining its host range (Liu et al. 2018a). Tn5 transposon mutants of USDA 110 were constructed, and 14 mutants were able to form normal nodules on *S. flavescens*, while wild-type USDA 110 only induced small pseudonodules with no bacteroids on this plant (Liu et al. 2018a). Analyses of the mutated genes found that they were involved in metabolism, transportation and chemotaxis and did not participate in the biosynthesis of Nod factors directly (Liu et al. 2018a). These results indicate that mutation of some nonessential genes could expand rhizobial host ranges (Brewin et al. 1980) and that promiscuity or specificity is dependent both on host and rhizobia (Liu et al. 2018a).

Ensifer adhaerens, a bacterial predator attacking and lysing *Micrococcus luteus* cells, was originally isolated from Hagerstown silty clay loam soil (Casida 1980, 1982). Controversy over the nomenclature of *E. adhaerens* and *Sinorhizobium adhaerens* is not our concern here, but *S. adhaerens* was previously isolated from root nodules of *Sophora tomentosa* grown in Brazil and Taiwan and China (Hung et al. 2005; Toma et al. 2017). Although *S.* (*E.*) *adhaerens* was reported to be a predominant occupant in nodules of *S. tomentosa* (Toma et al. 2017), the capability of nodulation and nitrogen fixation of this species need to be further checked carefully. Other endophytic bacterial species belonging to the genera *Bacillus*, *Brevibacillus* and *Paenibacillus* were also found within *S. tomentosa* nodules (Toma et al. 2017), further supporting the importance of nodulation tests with *S.* (*E.*) *adhaerens*.

*7.3.3 Rhizobia and Common Bean (***Phaseolus vulgaris***)*

As mentioned in Chap. 5, the promiscuous legume common bean (*Phaseolus vulgaris*) can be nodulated by various species mainly in the genus *Rhizobium*, including *Rhizobium etli* (Aguilar et al. 2004), *R. leguminosarum* (García-Fraile et al. 2010; Mulas et al. 2011), *R. lusitanum* (Valverde et al. 2006), *R. gallicum* (Amarger et al. 1997), *R. phaseoli* (Ramírez-Bahena et al. 2008), *R. tropici* (Amarger et al. 1994; Martinez-Romero et al. 1991), *R. leucaenae* (Ribeiro et al. 2012), *R. paranaense* (Dall'Agnol et al. 2014), *R. vallis* (Wang et al. 2011) and *R. sophoriradicis* (Jiao et al. 2015c; Ormeño-Orrillo et al. 2018), as well as *R. giardinii* (Amarger et al. 1997), which is now *Pararhizobium giardinii* (Mousavi et al. 2015). In addition, some isolates containing symbiosis genes (*nod* and *nif*) were found in the genera *Agrobacterium* (Wang et al. 2016), *Bradyrhizobium* (Cao et al. 2014) and *Ensifer* (Wang et al. 2016), and one non-nodulating species *Phyllobacterium endophyticum* (Flores-Félix et al. 2012) was reported to be isolated from root nodules of *P. vulgaris* grown in different soils of China, Mexico and Spain.

The phylogeny of *nodC* symbiosis genes of the rhizobia associated with common bean is discussed in Chap. 5 of this book.

*7.3.4 Bradyrhizobia and Peanut (***Arachis hypogaea***)*

Most of the effective rhizobia associated with peanut (*Arachis hypogaea*) belong to the genus *Bradyrhizobium* (Chen et al. 2003, 2016; El-Akhal et al. 2009; Santos et al. 2017; Taurian et al. 2006), though some effective fast-growing rhizobia have also been described (El-Akhal et al. 2009; Jaiswal et al. 2017; Osei et al. 2018; Taurian et al. 2006). *Bradyrhizobium arachidis* (Wang et al. 2013b), *B. guangdongense* and *B. guangxiense* (Li et al. 2015) have been described as novel species, and the type strains of these species were isolated originally from nodules of peanut. Strain CCBAU 23160, isolated from peanut grown in Anhui province, China, was identified as *B. lablabi*, though the type strain of *B. lablabi*, CCBAU 23086^T, was isolated from nodules of *Lablab purpureus* (Chang et al. 2011).

Bacteria from Ghana, closely related to *B. yuanmingense*, were reported to be effective peanut microsymbionts (Osei et al. 2018). In the same study, fast-growing isolates KNUST 1003 and 1007 were isolated that were highly similar to *Rhizobium tropici* (Osei et al. 2018). In another study, *Bradyrhizobium* isolates obtained from native peanut grown in Córdoba, Argentina, were classified as *B. iriomotense* and *B. yuanmingense* based on phenotypic and phylogenetic comparisons (Muñoz et al. 2011). Other isolates, also from Córdoba, were identified as *B. japonicum*, *Bradyrhizobium* sp., *R. gardinii* and *R. tropici* (Taurian et al. 2006).

Other potentially novel species of peanut symbionts are being described by researchers at China Agricultural University (personal communication). The symbiosis genes of peanut bradyrhizobia are described in Chap. 5 of this book.

7.3.5 *Bradyrhizobia and* Erythrophleum fordii

Erythrophleum fordii, commonly known as ironwood tree, is an evergreen legume in the subfamily Caesalpinioideae indigenous to the south of China and the north of Vietnam (Sein and Mitlöner 2011; Yao et al. 2014). Previously, only one rhizobial strain closely related to *B. elkanii* was reported to be a symbiotic bacterium of *E. fordii* (Lu et al. 2011). Later, systematic studies on the rhizobia of this ironwood tree were carried out by Yao et al. (2014, 2015).

A total of 166 bacterial isolates were obtained from the nodules of *E. fordii* growing in Guangdong and Guangxi provinces in southern China. All these isolates were found to be in the genus *Bradyrhizobium*, with 22 genotypes. Based on multilocus sequence analyses, five genospecies were identified: *B. elkanii* and *B. pachyrhizi* comprising the dominant symbionts and *B. yuanmingense*, *B. erythrophlei* and *B. ferriligni* comprising the minor symbionts (Yao et al. 2014, 2015).

7.4 Rhizobial Infection Through Root Hair Entry or Crack Entry

The establishment of symbiosis between legumes and rhizobia starts from the molecular recognition and interaction between them, followed by infection and entry of rhizobia into the leguminous cell. To form an efficient nitrogen-fixing nodule, live rhizobia must enter the root cell of legume through a specific pathway. Two major infection patterns can be found commonly: root hair infection thread (Fig. 7.4a) and crack entry (Fig. 7.4b). In addition, intercellular infection (Fig. 7.4c) was observed in some other legumes. Most legumes have only one of the infection patterns, while certain legumes can have two kinds of infection patterns.

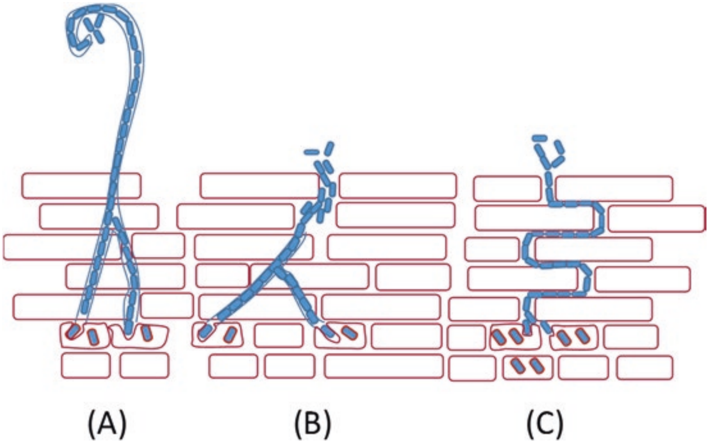

Fig. 7.4 Infection patterns. (**a**) Root hair infection thread. (**b**) Crack entry with or without infection threads. (**c**) Intercellular infection. The lower part of the figure shows endocytosis into cortical cells and the host-derived peribacteroid membrane surrounding the rhizobial bacteroids. (Figure redrawn from Madsen et al. (2010))

The root hair infection thread pattern (Fig. 7.4a) is found in most well-studied legumes, such as alfalfa (*Medicago sativa*) (Gage 2004), *Medicago truncatula* (Fournier et al. 2008), soybean (*Glycine max*) (Rao and Kerster 1978), pea (*Pisum sativum*), *Lotus japonicus* (Markmann et al. 2012; Xie et al. 2012) and *Sophora flavescens* (Jiao et al. 2015a). Rhizobial entry by the crack pattern (Fig. 7.4b) is found in certain legumes including peanut (*Arachis hypogaea*) (Boogerd and vanRossum 1997), joinvetch (*Aeschynomene americana*) (Grant and Trese 1996) and *Adesmia bicolor* (Bianco 2014), as well as the nonlegume, *Parasponia andersonii* (Bender et al. 1987). Besides these two major patterns of infection, other distinct patterns are observed in some legumes. A woody legume, tagasaste (*Chamaecytisus proliferus* ssp. *proliferus* var. *palmensis*), forms N_2-fixing indeterminate nodules in response to infection by strains of *Bradyrhizobium* sp. (*Chamaecytisus*) (Vega-Hernandez et al. 2001). The infection process in tagasaste starts with deformed root hairs, but the infection aborts early, and, instead, the rhizobia use the crack entry mode to move along the intercellular route, eventually entering the altered cell walls directly (Vega-Hernandez et al. 2001). In *Lotus japonicus*, two alternative intercellular infection modes, crack entry and infection thread independent single cell infection, were discovered using symbiotic mutants (Markmann et al. 2012). Similar to tagasaste, the semiaquatic legume *Sesbania rostrata* is also infected via an intercellular invasion; the bacteria colonise epidermal fissures at lateral root bases and trigger cortical cell death for infection pocket formation and subsequent intercellular and intracellular infection thread progression towards the primordium (Capoen et al. 2010).

In a study of the infection pattern of *Sophora flavescens* by different rhizobial strains (Fig. 7.5), the authors found the distinguished deformation of the root hairs infected by different rhizobia, leading to different morphologies (Fig. 7.5b–h) (Jiao et al. 2015a). Though different deformed root hairs, the rhizobia invade the root through infection thread clearly (Fig. 7.5h).

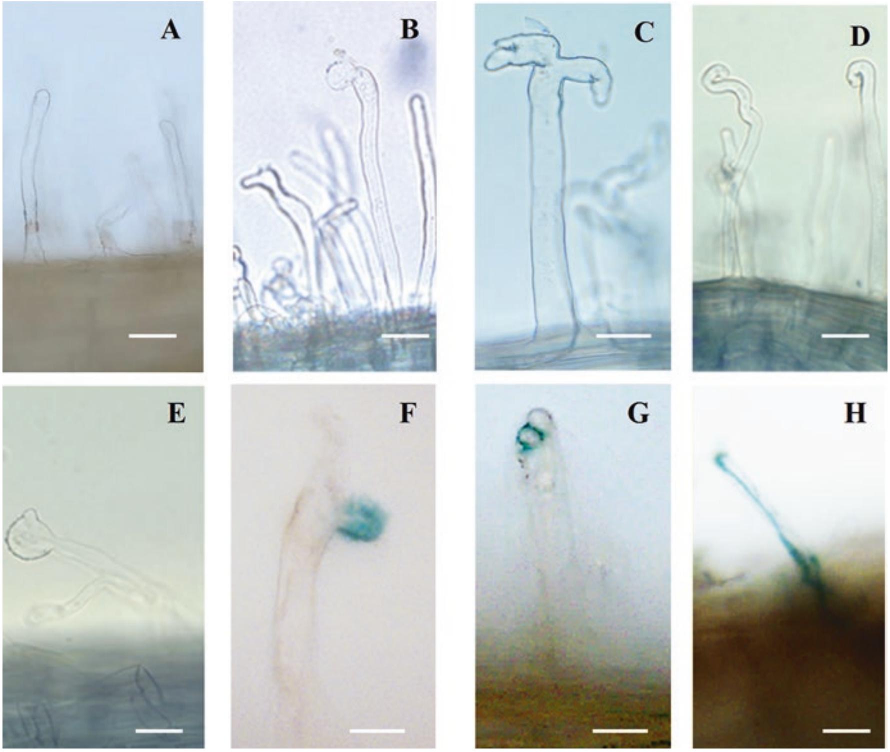

Fig. 7.5 Light micrograph of root hair, infection thread and nodule primordium of *Sophora flavescens* inoculated by different rhizobia (Jiao et al. 2015a). (**a**) Uninoculated root hairs at 6 days. (**b** and **c**) Root hairs, 6 days after inoculation (DAI) with *Sinorhizobium fredii* CCBAU 45436. (**d** and **e**) Root hairs, 6 DAI with *Rhizobium yanglingense* CCBAU 01603. (**f**) Aggregating cells of the *lacZ*-labelled *Sinorhizobium fredii* CCBAU 45436 surrounding the tip of a biforked root hair, 10 DAI. (**g**) Infection pocket formed by the *lacZ*-labelled *R. yanglingense* CCBAU 01603, 10 DAI. (**h**) Infection thread induced by *lacZ*-labelled *Sinorhizobium fredii* CCBAU 45436, 11 DAI. Bars: 40 μm in (**a**), (**b**), (**d**), (**e**) and (**h**); 20 μm in (**c**), (**f**) and (**g**)

7.5 Determinate or Indeterminate Root Nodules

Two major types of nodules, indeterminate (Fig. 7.6a, b) and determinate (Fig. 7.6c, d), are found on the roots of legumes. Types of nodules are classified according to their mode of development. The indeterminate type is characterised by a persistent nodule meristem (Fig. 7.6b), while the determinate nodule type lacks such a meristem (Fig. 7.6d). Therefore, indeterminate nodules are often clavate and elongated, while determinate nodules are usually spherical and have limited size.

Determinate nodules are found on root of cowpea (*Vigna unguiculata*) (Fig. 7.6c, d), soybean (*Glycine max*), peanut (*Arachis hypogaea*), mungbean (*Vigna radiata*), common bean (*Phaseolus vulgaris*), *Lotus japonicus*, etc. Legumes having indeterminate nodules include alfalfa (*Medicago sativa*), *Medicago truncatula*, white clover (*Trifolium repens*), red clover (*Trifolium pratense*), common vetch (*Vicia sativa*), broad bean

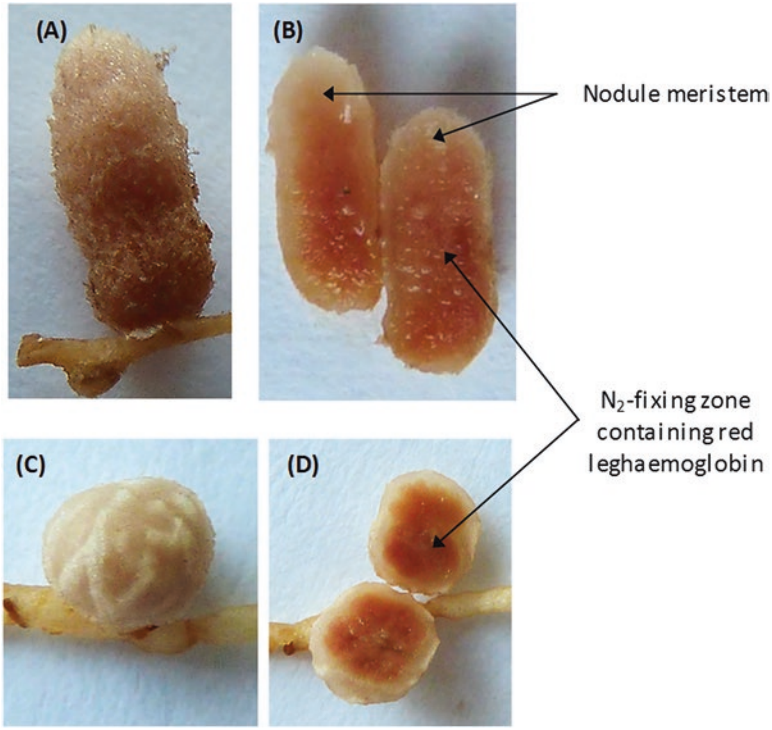

Fig. 7.6 Determinate and indeterminate nodules. (**a** and **b**) Indeterminate nodules of *Leucaena leucocephala*; (**c** and **d**) determinate nodules of cowpea (*Vigna unguiculata*). (**b**) and (**d**) are nodule sections showing the dark red N_2-fixing zone containing leghaemoglobin

(*Vicia faba*) and *Leucaena leucocephala* (Fig. 7.6a, b), pea (*Pisum sativum*) and *Sophora flavescens* (Jiao et al. 2015a, d). The type of nodule is determined by the host plant, not by the rhizobial strain (itself). The phenomenon is well demonstrated by the observation of root nodules of *S. flavescens* that only indeterminate nodules are formed regardless of the different rhizobial strains originally isolated from determinate nodule-forming legumes or the indeterminate nodule-forming legumes (Jiao et al. 2015a, d). Club-shaped pseudonodule, that no rhizobial bacteroid inside the nodule, could produce by infection of wild-type *Bradyrhizobium diazoefficiens* (formerly *Bradyrhizobium japonicum*) USDA 110 on *S. flavescens* (Liu et al. 2018b). Also nodule could initialise and develop induced only by the existence of Nod factor, lipooligosaccharide (LCO) (Stokkermans and Peters 1994). Therefore, the initiation of nodule primordium could be independent on the alive rhizobia but is dependent on LCO of rhizobia (D'Haeze and Holsters 2002). Another study showed that even without the LCO as well as rhizobia, the mutants of *Lotus japonicus* could form spontaneous nodules (Tirichine et al. 2006).

The biology of these two types of nodules has been fully described in a previous Tansley review (Hirsch 1992). Readers could refer this review for the comparison and differences of these two types of nodules.

7.6 Swollen or Non-swollen Bacteroids

Inside the legume nodule cells, rhizobia differentiate into N_2-fixing bacteroids which are physiologically and morphologically different from the free-living bacteria. Two kinds of bacteroids can be recognised: terminal or swollen bacteroids and nonterminal or non-swollen bacteroids. The size and shape of non-swollen bacteroids are similar to those of free-living bacteria, and these bacteroids can reproduce when they are re-isolated from the nodule cells. On the other hand, the swollen or terminal bacteroids have lost their reproductive capacity and no longer divide normally when they are re-isolated from the nodules (Zhou et al. 1985).

Typically, the differentiation of bacteroids to swollen or non-swollen types is determined by the host plant. Swollen/terminal bacteroids are observed usually in nodules of pea (*Pisum sativum*) (Oono and Denison 2010), alfalfa (*Medicago sativa*), peanut (*Arachis hypogaea*) (Fig. 7.7a, c) and clover (*Trifolium repens*) (Zhou et al. 1985). Branched, “Y”-shaped terminal bacteroids are found in pea, and

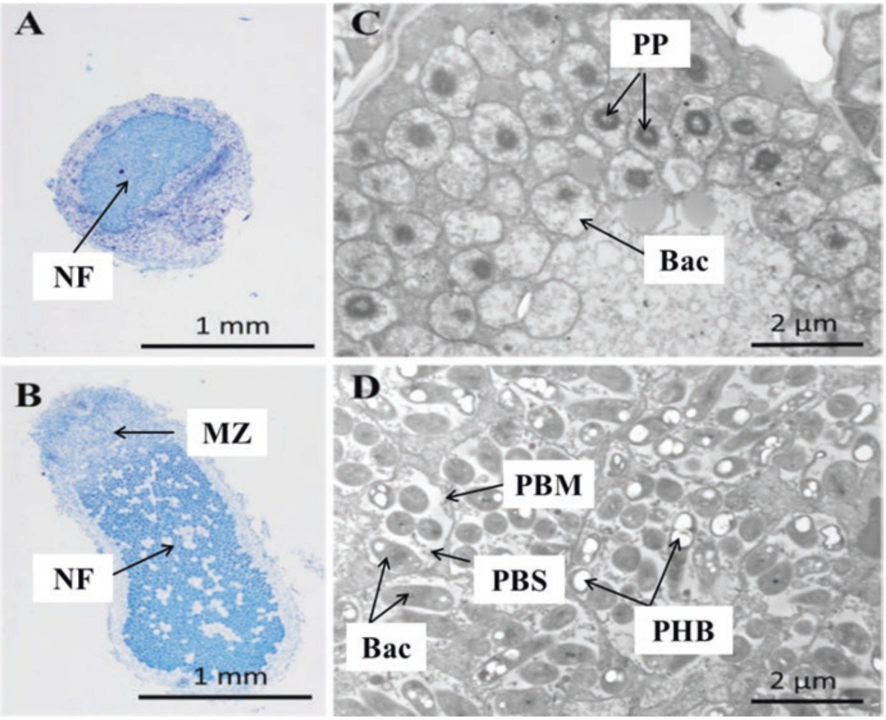

Fig. 7.7 Nodule section and transmission electron microscope of ultrathin nodule sections photographed by Xiang-Fei Meng. General light microscope of nodule sections of *Arachis hypogaea* (**a**) and *Sophora flavescens* (**b**). Transmission election microscope of ultrathin nodule sections of *Arachis hypogaea* (**c**) and *Sophora flavescens* (**d**). *Bac* bacteroid, *PBS* peribacteroid space, *PBM* peribacteroid membrane, *PP* electron-opaque polyphosphate inclusions, *MZ* meristem zone, *NF* nitrogen fixation zone, *PHB* poly-β-hydroxybutyrate

spherical bacteroids (Fig. 7.7c) are found in peanut. Non-swollen/nonterminal bacteroids are observed in nodules of soybean (*Glycine max*) (Zhou et al. 1985), bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*) (Oono and Denison 2010) and *Sophora flavescens* (Fig. 7.7b, d).

Swollen bacteroids have higher fixation efficiency as well as greater benefit to the legume host than non-swollen ones (Florian et al. 2018; Oono and Denison 2010).

7.7 Concluding Remarks and Perspectives

In this chapter, we have reviewed the diversity of interaction between rhizobia and legumes. Some rhizobia, like NGR234, can nodulate more than a hundred genera of legumes. However, this strain cannot nodulate some common leguminous crops, such as soybean, peanut, alfalfa and chickpea. Chickpea selects only *Mesorhizobium* with a specific *nodC* gene as its effective microsymbionts. In contrast, *Sophora flavescens* is an extremely promiscuous legume and can nodulate with different genera of rhizobia in the *Alphaproteobacteria* and *Betaproteobacteria*. The infection pattern, nodule and bacteroid development are also diverse in different symbioses.

With further investigation of the relationship between rhizobia and legumes, more novel rhizobia will be discovered. The range of symbiotic relationships among them may become more complex, but it will be illuminated more clearly.

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