Chapter 5
Genetic Diversity of Soybean Root Nodulating Bacteria

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Abstract Soybean (*Glycine max* L. Merrill), is a summer annual herb grown extensively in China, Argentina, Brazil and USA. It was thought to be very selective for its symbiotic partner. Earlier, only *Bradyrhizobium japonicum* was reported to nodulate soybean. It now is reported to be nodulated by a number of rhizobial genera and species; *Rhizobium, Bradyrhizobium, Mesorhizobium* and *Sinorhizobium*. Sinorhizobial species (*fredii*) nodulating soybean shows broad host range, where as the slow growing *Bradyrhizobium* is more selective. Slow growing *B. japonicum, B. elkanii, B. liaoningense* and *B. yuanmingense* are more effective nodulaters of soybean. Fast growers *Rhizobium tropici, Rhizobium* sp., *Rhizobium oryzae* and *Mesorhizobium tianshanense* have also been reported to form nodules on soybean. A large genetic diversity exists within the slow growing *Bradyrhizobium* isolates nodulating soybean. Due to the ecological and economic importance, the *Bradyrhizobia* species and their diversity have been extensively investigated in the last few years. The diversity and the size of indigenous population in soil can vary with the presence of the host legume and the history of the land use pattern at the sampling site. This review focuses on the genetic diversity existing in the bacteria nodulating soybean with special reference to Indian work.

5.1 Introduction

The rhizobium–legume symbiosis has been widely studied as the model of mutualistic evolution and the essential component of sustainable agriculture as biological fixation is the main source of nitrogen for natural and agricultural ecosystems. In agriculture, the symbiosis of nitrogen-fixing bacteria, collectively known as rhizobia, with crops belonging to the family Leguminosae (Fabaceae) are the most studied. Relatively high contributions to nitrogen nutrition have been demonstrated in pulses, fodders, green manures and trees. Members of the genus *Bradyrhizobium* constitute an important group of rhizobia, some of which form symbioses with economically important crops, such as soybean [*Glycine max* (L.) Merr.].

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Soybean (*Glycine max* L. Merr) is an annual herbaceous plant with its probable centre of origin and domestication in East Asia (Gai et al. 2000; Xu et al. 2002; Abe et al. 2003; Zhao and Gai 2004). For centuries soybean has been grown in China, Japan, Korea, Manchuria, the Philippines and Indonesia for various purposes and has been lovingly referred to as 'Cow of the field', or 'Gold from soil'. India is the fifth largest producer of soybean in the world next to United States, Brazil, Argentina and China. In India, Madhya Pradesh also known as the 'Soy State' alone contributes 56–67 % in total area and production of soybean. About 97 % of total area and 96 % production of soybean in the country come from the three states of Madhya Pradesh, Maharashtra and Rajasthan.

Soybean is a major source of vegetable oil, protein, animal feed and is also used for improving soil nutrient status. It is the seventh most harvest crops of the world by tonnage. Soybean alone is estimated to produce up to 200 kg N ha^{-1} in aboveground biomass in a single growing season. Of the soybean nitrogen content, 58–68 % is estimated to be derived from symbiotic nitrogen fixation (Salvagiotti et al. 2008; Peoples et al. 2009; Jensen et al. 2012). Following harvesting, the remaining portions of the plant, including roots and nodules which represent 30–60 % of the nitrogen content, are left to replenish the nitrogen content of the surrounding soil. Soybean contains about 22 % essential amino acids, over 40 % proteins and about 22 % oil. Soybean oil contains about 85 % unsaturated fatty acids (Malik et al. 2006) making it among the lowest of the vegetable oils. It is also rich in Omega 3 and Omega 6 fatty acids essential for regulating lipid and cholesterol metabolism. Soybean is reported to have several health benefits such as lowering cholesterol (Anderson et al. 1995; Van Horn et al. 2008), suitable dietary option – for type 2 diabetes due to its mostly starch free carbohydrates (Holt et al. 1996) and for the prevention and control of obesity (Velasquez and Bhathena 2007).

5.2 Soybean—*Bradyrhizobium* Symbiosis

Like any other legume soybean forms symbiotic partnership with Gram-negative α–Proteobacterial members, viz., *Sinorhizobium (Ensifer)* and *Bradyrhizobium*. Soybean forms determinate nodules which lose meristematic activity shortly after initiation, thus growth is due to cell expansion resulting in mature nodules which are spherical in shape. When Fred et al. (1932) proposed seven cross inoculation groups for categorizing root nodule bacteria, soybean was found to nodulate with only one genus and species; *Rhizobium japonicum*. However, in time it was observed that the legume could form effective symbiotic relationship with more genera and species.

5.2.1 Root Exudates and Molecular Signaling

The inter-recognition via chemical signals between a host plant and rhizobium is highly specific for both species and is indispensable for the development of

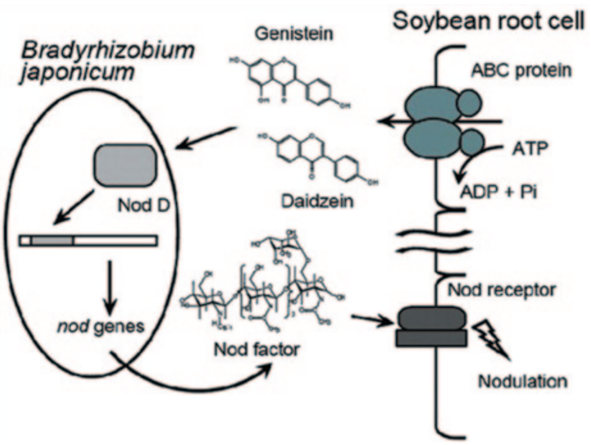

Fig. 5.1 A model of flavonoid secretion from soybean roots and the inter-recognition between legume and rhizobium. (Sugiyama et al. 2008)

functional nodules. These sophisticated signaling cascades begin with the secretion of flavonoids from the roots of the host plant, which are recognized by the NodD protein in the rhizobia leading to the successive induction of *nod* gene expression to produce the second signaling molecules, lipochitooligosaccharides, also known as Nod-factors (Fig. 5.1) (Sugiyama et al. 2008). Nod-factors perceived by receptors in the host plant induce various signaling events such as 'calcium spiking' response, ultimately resulting in the formation of nodules. As signaling flavonoids, isoliquiritigenin, genistein and daidzein were identified in root exudates of soybean (Kosslak et al. 1987; Smit et al. 1992). However, it is still not clear how the same host genotype producing these two specific flavonoids genistein and daidzein can recruit different rhizobial genera to form effective partnership. Obviously, there must be something common produced by the rhizobial species which is the main recognition factor for the flavonoid secreted by the plant. However, comparative genomics of *Sinorhizobium fredii* and *B. japonicum* hasn't shown any gene common or specific to these soybean microsymbionts compared with other legume microsymbionts. This shows that there is no gene specifically shared by *Sinorhizobium* and *Bradyrhizobium* to establish symbiosis with soybean (Tian et al. 2012).

5.2.2 Nodule Development

Nodulation involves the intimate relationship of *Bradyrhizobium*/*Sinorhizobium* and soybean plants, which results in the formation of a novel organ, the nodule, in which the bacteria reside and provide a steady source of nitrogen to the plant. Nodule formation requires nodule organogenesis and bacterial infection which must be both spatially and temporally coordinated. The two processes requires multiple and complex chemical cross-talk and coordinated expression of several genes (Oldroyd et al. 2011). Primarily through an examination of plant mutants defective in nodulation, the basic steps in the Nod factor signaling pathway have been elucidated

(Oldroyd and Downie 2008). Initial Nod factor recognition is mediated through two LysM receptors that likely directly interact with the compatible Nod factor (Amor et al. 2003; Madsen et al. 2003; Radutoiu et al. 2003). The resulting signaling cascade ultimately leads to activation of specific transcription factors (TFs), a few of which have been identified.

Perception of Nod factors by the receptors GmNFR1 and GmNFR5 in soybean roots (Indrasumunar et al. 2010, 2011) triggers many of the early events in nodulation including ion fluxes, root hair curling and root cortical cell division (Denarie et al. 1996; Kamst et al. 1998; Hirsch and Oldroyd 2009). The recognition of nod factors leads to nucleolar calcium spiking and the subsequent activation of the symbiotic signaling pathway which requires symbiosis receptor-like kinase (SYMRK) (Endre et al. 2002). Calmodulin dependent protein kinase (CCaMK) (Levy et al. 2004; Mitra et al. 2004; Tirichine et al. 2006) and a protein of unknown function (CYCLOPS) (Yano et al. 2008) are needed for perception of calcium spiking. This further initiates downstream signaling events including expressions of several transcription factors (nodulation signaling pathway [NSP]1, NSP2, ERF required for nodulation [ERN]1, nodule inception [NIN]) that regulate NF-induced gene expression and subsequently leading to nodule morphogenesis (Schauser et al. 1999; Kalo et al. 2005; Smit et al. 2005; Andriankaja et al. 2007; Marsh et al. 2007; Oldroyd et al. 2011). Soybean root nodules are of the ‘determinate’ type as they arise from the central cortex and have a transient meristem (Ferguson et al. 2010). Typically, soybean nodules senesce after few weeks and they are eventually replaced by developing new nodule structures.

In order to limit loss of carbon through excessive nodulation and also to maintain the balance in the symbiosis association, nodulation in soybean is tightly regulated through the Autoregulation of Nodulation (AON) pathway. The AON pathway operates through long distance signaling between the root and shoot. In soybean, GmRIC1 and GmRIC2 which are CLV3/ESR-related (CLE) peptide hormones (Lim et al. 2011; Reid et al. 2011; Reid et al. 2013) are synthesized in the roots and then translocated to the shoots via xylem (Delves et al. 1986; Reid et al. 2011) where they are recognized by the LRR receptor kinase, GmNARK/LjHAR1/MtSUNN/PsSYM29 (Krusell et al. 2002; Searle et al. 2003; van Hameren et al. 2013). In soybean, kinase-associated protein phosphatases, *GmKAPP1* and *GmKAPP2* are believed to be working in conjunction with the LRR receptor kinase, GmNARK in the signal transduction pathway (Miyahara et al. 2008; Ferguson et al. 2010). Shoot-Derived Inhibitor (SDI) is then produced and translocated to the root via the phloem upon perception of the CLE signal which eventually inhibits further nodulation (Ferguson et al. 2010; Reid et al. 2011). Several other genes including *LjKLAVIER* (Oka-Kira et al. 2005), ERF transcription factor, *MtEFD* (Vernie et al. 2008), and *LjASTRAY* (Nishimura et al. 2002) are known to regulate nodule numbers. Nodulation is also regulated by various environmental stresses such as acidity and soil nitrate (Carroll et al. 1985; Lin et al. 2012). All in all nodule development is regulated both by the genetic makeup of the host as well as environmental cues.

5.3 Genetic Diversity of Root Nodule Symbionts

The most important factor of nodule formation is the prokaryotic symbiont. Soybean (*Glycine max*) is known to be nodulated by at least five species of rhizobia belonging to two different genera with almost all the species reported from China. These species includes both the slow growing and fast growing rhizobia with diverse morphological, biochemical, physiological, genetic and symbiotic attributes. Generally, fast growing rhizobia are classified as those bacteria having generation of about 1.5–4 h and produces acid on YEM (Yeast Mannitol Medium). Slow growing bradyrhizobia on the other hand, have a generation time of at least 6 h and produces alkali on YEM medium (Table 5.1). *Bradyrhizobium japonicum, B. elkanii, B. liaoningense, B. yuanmingense* are the slow growing rhizobia nodulating soybean and *S. fredii* the fast growing symbiont (Man et al. 2008; Vinuesa et al. 2008; Han et al. 2009; Li et al. 2011a, b; Zhang et al. 2011). Clear biogeography of soybean rhizobia has been shown in China (Han et al. 2009; Man et al. 2008; Wang et al. 2009). However, less is known about soybean's nodule microsymbionts in an ecological context despite its long history of cultivation and wide distribution across Indian continent.

5.3.1 Fast Growing Isolates

The characterization of the indigenous fast-growing symbionts of soybean root nodules is important to understand better the ecological strategies that tropical rhizobia may take to survive after the introduction of an exotic host legume. In (1982) fast-growing rhizobial strains were isolated from soybean nodules and from soil of the People's Republic of China, within the center of origin and diversity of this legume (Keyser et al. 1982). Later, fast-growing strains were isolated from other primary and secondary centers of soybean origin (Xu and Ge 1984; Dowdle and Bohlool 1985; Rodriguez-Navarro et al. 1996). These fast growers were classified as the new species *Rhizobium fredii* (Scholla and Elkan 1984), later reclassified as *S. fredii* and *S. xinjiangensis* (Chen et al. 1988), and recently proposed to change to the genus *Ensifer* (Young 2003). Although it was originally thought that *S. (Ensifer) fredii* was specific for Asian soybean lines (Keyser et al. 1982; Stowers and Eaglesham 1984), later it has been shown that several North American and Brazilian genotypes are capable of forming effective nodules with those bacteria (Balatti and Pueppke 1992; Chueire and Hungria 1997). Based upon phenotypic characterization and DNA–DNA hybridization (Chen et al. 1988; Peng et al. 2002), two species of fast growers *S. fredii* and *S. xinjiangensis* were proposed, however, *E. xinjiangensis* has subsequently been incorporated into *S. fredii* based upon multilocus sequence analysis (Martens et al. 2008).

In recent times genetic diversity in *S. fredii* strains have been observed by different workers. Their work has categorized fast growing strains nodulating *G. max* into five genomic groups; *S. fredii*, *Sinorhizobium* sp. I, *Sinorhizobium* sp. II,

Table 5.1 Diversity of root nodulating bacteria isolated from soybean

Strains	Generation time	Geographic origin of strains	Colony size (mm)	G+C (%)	References
Bradyrhizobium japonicum	>6 h	Ibaraki, Japan	1.0[c]	61–65	Jordan (1982)
B. elkanii	>6 h	China	1.0[c]	62–64	Kuykendall et al. (1992)
B. liaoningense	16.4–39.6 h	Liaoning Province, China	0.2–1.0[c]	60–64	Xu et al. (1995)
B. yuanmingense	9.5–16 h	China	1.0–2.0[c]	62–64	Yao et al. (2002)
Sinorhizobium (Ensifer) fredii	3–4 h	China	2.0–4.0[a]	60–64	Chen et al. (1988)
B. huanghuaihaiense sp. nov.	7–9 h	Huang-Huai-Hai, China	1.0[c]	61.5	Zhang et al. (2012)
B. daqingense sp. nov.	10 h	Daqing city, Heilongjiang province, China	1.0***	61.2	Wang et al. (2013)
E. sojae	3–4 h	Hebei province, China	1.0–3.0[a]	60.9	Li et al. (2011b)
E. xinjiangense	3–4 h	Xinjiang Province, China	2.0–4.0[a]	60.1–60.9	Chen et al. (1988) Peng et al. (2002)
Mesorhizobium tianshanense	Variable generation time	Xinjiang, China	1.0–2.0[b]	59–63	Chen et al. (1995)
R. tropici	1.6–2 h	Brazil	2.0–4.0[d]	59–65	Hungria et al. (2006)
Rhizobium sp.	2–3 h	Brazil	2.0–3.5[d]	59–65	Hungria et al. (2006)

[a] After 3–4 d of incubation

[b] After 5–7 d of incubation

[c] After 7–10 d of incubation

[d] After 2–4 d of incubation

Sinorhizobium sp. III and *S. sojae*. Different levels of genetic differentiations were observed among these species. *S. sojae* was most divergent from the other test species and was characterized by its low intraspecies diversity and limited geographic distribution. There was no geographic isolation between *S. fredii* populations in different ecoregions in China (Guo et al. 2014).

5.3.2 Slow Growing Isolates

The slow growing root nodulating bacterium of soybean was initially termed *Rhizobium japonicum* which was later delineated into a separate genus *Bradyrhizobium* because of its slow growth. The slow growers able to form effective root nodules on soybean are distributed in four species of the *Bradyrhizobium* genus, namely, *Bradyrhizobium japonicum*, *Bradyrhizobium liaoningense, B. yuanmingense* and *Bradyrhizobium elkanii.*

Multilocus sequence analysis of the soybean rhizobia in the Asiatic Continent (Myanmar, India, Nepal, and Vietnam), revealed the presence of all the four *Bradyrhizobium* species viz*., B. japonicum, B. elkanii, B. liaoningense,* and *B. yuanmingense. B. japonicum* and *B. elkanii* were found to be dominant in the humid and temperate climates of the Northern hemisphere, *B. liaoningense* in the East and Southeast while *B. yuanmingense* from Northern to Southern hemisphere with climatic conditions ranging from humid equatorial or dry, hot and semiarid (Vinuesa et al. 2008; Appunu et al. 2008). Of particular interest is *B. yuanmingense,* a very promiscuous symbiont with very broad geographic and host ranges capable of nodulating besides soybean, *Lespedeza* sp. (Vinuesa et al. 2005a), lima beans (Ormeno-Orrillo et al. 2006), *Indigofera hirsute* (van Berkum et al. 2003) and *Vigna* species (Zhang et al. 2008). Vinuesa et al. (2005b) however suggest the presence of symbiotic ecotypes within this species as strain isolated from one host do not cross nodulate the other. In the different agro-eco-climatic soybean growing regions of India with conditions varying from hot, sub humid and neutral to slightly acidic soils to hot, semiarid and highly alkaline soils three major species viz., *B. japonicum, B. yuanmingense,* and *B. liaoningense* and rarely *Sinorhizobium* sp. were found to be the main symbiont of soybean (Annapurna et al. 2007; Appunu et al. 2008, 2009). Soils of the temperate region of Nepal were found to be dominated by *B. japonicum* while in the subtropical regions with acidic, moderately acidic, and slightly alkaline soils; *B. elkanii, B. yuanmingense, and B. liaoningense* were the dominant symbionts (Adhikari et al. 2012).

In the American Continent, *B. japonicum* and *B. elkanii* have been reported to be the dominant species nodulating soybean. Genetic diversity studies of soil samples taken from acidic to slightly alkaline soybean fields across nine different states in the United States revealed the prevalence of *B. japonicum* and *B. elkanii* (Shiro et al. 2013). Similar observations were also reported from Brazil (Giongo et al. 2008) and Paraguay (Chen et al. 2000). In Brazil, however, only *B. japonicum* and *B. elkanii* have been used as commercial inoculants to increase soybean yields. Since the Brazilian soils lacks indigenous soybean bradyrhizobia (Alberton et al.

2006), its entire naturalized bradyrhizobia population nodulating soybean possibly came with seeds and inoculants from United States.

In India, where soybean was introduced in 1960s, native rhizobial populations slowly grew and segregated. Initially the inoculant was a strain from USA, the slow growing *B. japonicum*. Over the years as soybean was cultivated in various parts of the country, with indigenous cultivars, soybean rhizobial population developed. Dominant group still seems to be slow growers *B. japonicum*, B. *liaoningense* and *B. yuanmingense* (SatyaPrakash and Annapurna 2006; Appunu et al. 2008), though fast growing soybean nodulating rhizobia have also been reported from the country.

Interestingly, slow growing bradyrhizobia nodulating soybean comprises a heterogeneous group (Menna et al. 2009; Delamuta et al. 2012, SatyaPrakash and Annapurna, 2006; Tian et al. 2012). Molecular characterization using genetic typing methods like rep-PCR, 16S-RFLP, *nif*H and *nod*C, and MLSA were carried out to analyze the genetic diversity of bradyrhizobia nodulating soybean by these workers. Several genospecies have been reported within the group (Appunu et al. 2008; Man et al. 2008; Han et al. 2009). This in part could be attributed to the existence of transfer of symbiotic genes within the group. Vertical transfer by and large is the main phenomena for transfer of symbiotic genes within the group (Man et al. 2008; Han et al. 2009). Lateral transfer of symbiotic genes have also been reported (Han et al. 2009) while horizontal transfer of symbiotic genes is a very rare occurrence within the group. Interestingly, Marchetti et al. (2010) observed that transfer of *nod-nif* genes from the rhizobium *Cupriavidus taiwanensis* enable the pathogenic *Ralstonia solanacearum* to infect and nodulate *Mimosa* sp. which evidently points to the importance of *nod-nif* genes and gene activation in the evolution of bacteria into rhizobia. Tian et al. (2012) further argued that in addition to vertical and lateral transfer of genes, rhizobia also recruits lineage specific genes through symbiotic interactions and environmental adaptations.

Among a few studies on soybean rhizobial diversity in Nepal, some genetically distinct *B. japonicum* strains compared to those in other Asian countries have been reported in a soil in the Kathmandu Valley (Vinuesa et al. 2008). Recently, the diversity of soybean bradyrhizobia was assessed in five mountain soils of Nepal ranging from 1500 to 2600 m in elevation with soil pH levels ranging from 5.2 to 6.2, and a dominant presence of *B. elkanii* with minor populations of *B. japonicum* and *B. yuanmingense* was reported (Risal et al. 2010).

Besides these group of well documented rhizobial symbionts, *Rhizobium tropici, Rhizobium* sp. (Hungria et al. 2006), *Rhizobium oryzae* (Peng et al. 2008), *Mesorhizobium tianshanense* (Chen et al. 1995) have also been reported from the root nodules of soybean.

5.3.3 Biogeography of the Root Nodule Symbionts

Differentiated rhizobial gene pools nodulating *G. max* have been reported in different ecoregions of China. Rhizobia nodulating soybean are known to exhibit biogeographic pattern with soil pH and temperature playing a dominant role in influencing their genetic diversity and distribution (Man et al. 2008; Han et al. 2009; Zhang et al. 2011). Soil nutrients including available P, K and N also effect the distribution and diversity of soybean rhizobia. In the soybean growing soils of North China Plain, high N concentration favored *S. fredii, B. liaoningense,* and *B. yuanmingense* population while it had a negative effect on the *B. elkanii* (Zhang et al. 2011). Positive correlation between high content of available K and growth of *B. yuanmingense* was observed in Hebei Province of China while the same inhibited *B. japonicum, B. elkanii* (Li et al. 2011a, b). The authors also reported correlation between high available P in the soil and the distribution of rhizobia in the same region nodulating soybean. Broadly speaking, *Sinorhizobium* is dominant in alkaline-saline soil whereas *Bradyrhizobium* in neutral to acidic soil (Man et al. 2008; Vinuesa et al. 2008; Han et al. 2009; Li et al. 2011a, b; Zhang et al. 2011). In the subtropical and tropic regions of China with humid and acid soils soybean preferred *B. japonicum, B. elkanii* over *B. liaoningense,* and *B. yuanmingense* as symbionts (Yang et al. 2006; Man et al. 2008). In the humid climate of Northeast China having neutral to slightly acidic soils the dominant symbiont of soybean was *B. japonicum* but never *S. fredii* (Wang et al. 2009). Sawada et al. (1989) examined 85 Japanese indigenous soybean-nodulating rhizobial strains isolated from 46 soils around Japan for their hydrogenase uptake (Hup) trait and somatic serogroup identity and suggested a relationship between the distribution of serogroup and Hup phenotype strains. Minamisawa et al. (1999) examined 213 indigenous soybean bradyrhizobia isolated from six fields in Japan by fingerprint analysis with probes for RSα, RSβ, *nifDK* and *hupSL*, and suggested that the diversity of bradyrhizobia in individual fields is associated with host plants and local soil conditions. Similar studies are lacking in India. No rhizobial map has been generated for soybean growing areas in the country. SatyaPrakash and Annapurna (2006) gave the first report on the genetic polymorphism existing in root nodulating bacteria from soybean grown in one field. Root nodule isolates from the four varieties were *B. japonicum* types, growing in 4–7 days with typical colonies which were found to be genetically distinct from the USDA and SEMIA strains of *B. japonicum* and *B. elkanii.* Appunu et al. (2008) showed that the diversity is wider than expected based on previous studies in various geographic areas and on the current taxonomy of soybean rhizobia. Notably, the diversity of the soybean symbionts appears to be conserved across the agricultural-ecological-climatic regions sampled. In recent times we have come across fast growing rhizobia able to nodulate Indian cultivars of soybean (unpublished).

How could bacteria of these two contrasting genera (*Bradyrhizobium* and *Sinorhizobium*) evolve into the microsymbionts of the same legume plant? Recent comparative genomics of soybean rhizobia revealed that the core genome of *Bradyrhizobium* is rich in lipid and secondary metabolism genes whereas the *Sinorhizobium* core genome had several gene clusters known to be involved in osmoprotection and adaptation to alkaline pH, corroborating the biogeographic pattern of distribution of soybean rhizobia (Tian et al. 2012) (Table 5.2). However, it would be very interesting if we could find out specific genes involved for recognition/nodulation in these two genera. For this we need to sequence a large number of genomes from each genus (*Bradyrhizobium* and *Sinorhizobium*) to reveal any distinct feature specific for each genus related to their symbiotic capacity and environmental adaptations

Table 5.2 Comparison of COG assignments between *Sinorhizobium* and *Bradyrhizobium* nodulating soybean. (Courtesy Tian et al. (2012). Some selected functional categories where distinct differences were noted. For more details please see the original reference)

Individual functional categories	Genus specific genes	
	Sinorhizobium	*Bradyrhizobium*
J: Translation, ribosomal structure and biogenesis	8	18
L: Replication, recombination and repair	9	16
V: Defense mechanisms	6	11
N: Cell motility	16	25
F: Nucleotide transport and metabolism	16	8
E: Amino acid transport and metabolism	75	97
I: Lipid transport and metabolism	16	54
C: Energy production and conversion	39	55
Q: Secondary metabolites biosynthesis, transport and catabolism	10	45
R: General function prediction only	75	113

5.4 Conclusion

Glycine max (soybean) is one of the most important legume crops in the world thought to have originated in East Asia. Rhizobia that nodulates soybean represent a heterogeneous group. At least five rhizobial species have been reported as its microsymbionts by independent studies. In India, where soybean is cultivated in more than 10 million ha a large native population has developed which needs to be studied for its genetic diversity. Earlier reports by researchers have indicated at the heterogeneity existing among the strains forming root nodules on different cultivars. As the crop is grown in different agro-climatic regions in the country, it would be interesting to bio-prospect these strains. Recent comparative genomic studies have revealed that there is no gene specifically shared by *Sinorhizobium* and *Bradyrhizobium* to establish symbiosis with soybean. However, a large genetic diversity exists in the symbionts nodulating soybean. Recombination seems to have contributed to this diversity of the core genome of these symbionts.

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