

Review Article

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Tree Legume - Rhizobia Symbiosis and its Potential as Biofertilizer: A Review

Sampi Romin^{ID} and Diptimayee Dash*

Department of Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur - 492012,
Chhattisgarh, India

*Corresponding author

ABSTRACT

Tree legumes, which are mainly found in the Caesalpinioideae, Mimosoideae, and Papilionoideae subfamilies of the Leguminosae family, are widely used in the restoration of degraded lands. As nitrogen-fixing trees, they are widely regarded as essential components of sustainable agroforestry systems, and their rhizobial symbionts can help revive severely degraded soils by accelerating natural ecological succession. Rhizobial biofertilizers offer a cost-effective, environmentally responsible alternative, reducing dependence on synthetic inputs while supporting ecosystem repair. A biofertilizer is broadly defined as a formulation of viable living microorganisms that improves soil fertility and plant growth, performing a role comparable to chemical fertilizers, making region-specific microbial strains an important research priority. This review examines the taxonomy and diversity of rhizobia associated with tropical and sub-tropical tree legumes, synthesizes existing literature on rhizobia and their plant growth-promoting (PGP) traits. Particular attention is given to studies characterizing rhizobial isolates from tree legume nodules across diverse agro-climatic regions and their potential application as commercial biofertilizers under regulatory quality standards. Considerable variation exists among rhizobial strains in terms of host range and symbiotic effectiveness; however, region-specific characterization of rhizobia associated with many tree legume species remains inadequate. Filling this knowledge gap is essential for the development of efficient, locally adapted biofertilizer formulations in the long run.

Keywords

Rhizobia; tree legumes; biological nitrogen fixation; plant growth-promoting traits; biofertilizer

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Introduction

Biological nitrogen fixation (BNF) offers a cost-effective and environmentally sound alternative for meeting crop nitrogen requirements without these drawbacks. Among BNF systems, the symbiosis between leguminous plants and rhizobia, a diverse group of soil bacteria capable of inducing nodule formation on plant roots or stems is the

most agriculturally significant. Due to its positive effects on soil fertility and crop output, rhizobia have become more popular as a biofertilizer in tropical and sub-tropical regions of India. Because of their ecological adaptability and the increasing demand for region-specific microbial inoculants in sustainable agriculture, the study of rhizobia associated with tree legumes, including their isolation, classification, and functional

assessment has become an important area of research. Consequently, rhizobial inoculants are emerging as promising biofertilizers for sustainable crop production, in reducing environmental pollution and in biological agri-business. This review provides a comprehensive overview of the symbiotic relationship between tree legumes and their associated rhizobia, highlighting their importance in sustainable agriculture, agroforestry, and ecosystem restoration. It focuses on three major aspects. First, it examines the evolving taxonomy of rhizobia and explores the diversity of rhizobial isolates associated with different tree legume species. Second, it discusses the plant growth-promoting characteristics of these isolates, including their ability to fix atmospheric nitrogen, solubilize phosphate, enhance nutrient availability, and tolerate environmental stresses. Finally, the review evaluates the potential of these rhizobial strains as biofertilizers, addressing their role in improving soil fertility, reducing dependence on chemical fertilizers, and supporting sustainable agricultural and agroforestry systems

Historical background of rhizobial classification

Beijerinck (1888) isolated the causative microorganism from legume root nodules and named it *Bacillus radicicola*; this organism was subsequently renamed *Rhizobium leguminosarum* by Frank (1889). The taxonomic placement of *Rhizobium* as currently recognized is summarized in Table 1. Despite the ecological importance of tree legumes, the rhizobial genera associated with their root nodules vary considerably across host species and geographic regions, as reported by various researchers (Beukes *et al.*, 2019; Martínez-Romero *et al.*, 1991; Moura *et al.*, 2020; Rasul *et al.*, 2012) is presented in Table 2.

Rasul *et al.*, (2012) worked on numerical taxonomy, 16S rRNA gene sequencing, and cross-nodulation assays to characterize their isolates. Their 16S rRNA sequence data showed that the majority of *M. pinnata* microsymbionts fell within the genera *Rhizobium* and *Bradyrhizobium*, showing close phylogenetic affinity to *Rhizobium* sp. and the species *B. elkanii* and *B. yuanmingense*.

Isolation and Characterization of Rhizobia

Isolation of rhizobia from tree legume root nodules typically follows standard protocols involving surface sterilization of nodules, plating on yeast extract–mannitol

(YEM) agar, and subsequent purification of single colonies. Dhiman *et al.*, (2018) recovered 79 bacterial isolates from nodules of *Dalbergia sissoo* across Himachal Pradesh and Uttarakhand using YEMA at 37°C, and through a combination of Congo red exclusion, bromothymol blue reaction, and plant-infection (authentication) tests, confirmed 38 of these as true rhizobia. All confirmed isolates displayed the characteristic Gram-negative, pink-staining, rod-shaped colony morphology typical of the genus, also reflected in the work of Niste *et al.*, (2015), who characterized 20 rhizobial isolates from red clover (*Rhizobium trifolii*) and alfalfa (*Sinorhizobium meliloti*) isolated 150 bacterial strains from nodules of *Prosopis juliflora* in the Marigat region of Kenya, of which 60 were subjected to detailed morphological, cultural, and biochemical characterization. The considerable physiological diversity observed among these strains supports earlier evidence that this host legume associates with a broad spectrum of nitrogen-fixing microsymbionts.

Diversity and host specificity of Rhizobia associated with tree legumes

Rhizobia associated with tree legumes exhibit varying degrees of host specificity. While some strains form symbiotic relationships with only a limited range of host species, others are capable of nodulating a wide variety of legumes across different genera. Boakye *et al.*, (2017) examined indigenous rhizobial populations nodulating five multipurpose tree legumes across three Ghanaian soil types. Isolates from *M. thonningii* proved highly host-specific, nodulating only their original host, whereas isolates from *L. leucocephala*, *Crotalaria ochroleuca*, and *Cajanus cajan* were moderately promiscuous. These tests indicate that the mere presence of nodulating rhizobia in soil does not guarantee effective symbiosis. A similar trend was observed by Li *et al.*, (2016) in their study of 198 rhizobial isolates associated with *Sesbania cannabina* growing in saline–alkaline soils. Using 16S rRNA sequencing and multilocus sequence analysis of housekeeping genes (*recA*, *atpD*, and *glnII*), the authors identified nine genospecies belonging to the genera *Ensifer*, *Agrobacterium*, *Neorhizobium*, and *Rhizobium*. Among these, *Ensifer* species were the most prevalent in both study regions. These findings suggest that the host plant strongly favors rhizobia possessing compatible symbiotic genes. In contrast, Bala and Giller (2001) found that rhizobial isolates from *Calliandra calothyrsus*, *Gliricidia sepium*, and *L. leucocephala* exhibited broad host promiscuity, readily cross-nodulating each other's

hosts as well as several additional legume species, while symbionts of *Sesbania sesban* displayed a comparatively narrow host range. Importantly, plants inoculated with effective rhizobial strains showed superior nitrogen use efficiency compared to those receiving mineral nitrogen alone. The benefits of combining rhizobial strains were directly demonstrated by Megu and Deb (2025), who tested three rhizobial isolates from wild *Crotalaria tetragona*, *C. mysorensis*, and *L. leucocephala* individually and in four consortium combinations against four edible crop legumes. Individual isolates varied widely in host range and effectiveness: MOKCS15 broadly, while LUMLL12 showed limited effectiveness. Importantly, combinations of rhizobial strains were generally more effective than individual strains, with the four-strain consortium showing the best performance, together with the findings of Boakye, which showed that many isolates were ineffective and different strains can complement each other's limitations. Junior *et al.*, (2017), surveying rhizobial diversity across Brazilian pulses, forage legumes, and tree legumes, concluded that soil and climatic factors, together with host genetic variation, strongly influence rhizobial community composition diversity. These studies show that, while some hosts, such as *M. thonningii*, exhibit strong specificity, others, including *C. calothyrsus* and *G. sepium*, are capable of forming associations with a broad range of rhizobial species.

Tree legumes and their ecological role in biological nitrogen fixation

Before atmospheric nitrogen can be assimilated by plants, it must be reduced to reactive nitrogen, such as ammonia, through either the Haber–Bosch process or biological nitrogen fixation by specialized bacteria and archaea (Buren & Rubio, 2017). Aryal *et al.*, (1999) evaluated the effect of *Rhizobium* inoculation on seedlings of *Albizia procera*, *Albizia lebbeck*, and *Leucaena leucocephala* and found increased nitrogenase activity by as much as 68.75-fold in sterilized soil and 6.7-fold in non-sterilized soil. A subsequent nursery survey by Aryal *et al.*, (2015) involving seven legume tree species further highlighted the potential of *L. leucocephala*. The species consistently outperformed all others, producing the highest number of nodules along with the greatest nitrogenase activity, seedling height, and root and nodule biomass. These findings suggest that *Rhizobium* inoculation is a key factor in maximizing nodule formation and enhancing the nitrogen-fixing capacity of *L. leucocephala*. Kohley (2012) conducted a

greenhouse study evaluated the effects of *Rhizobium* inoculation and different nitrogen levels on the growth of *Albizia procera* seedlings. The results showed that seedlings receiving *Rhizobium* inoculation combined with 150 mg N per seedling exhibited the best growth and biomass production, outperforming both uninoculated and singly treated plants. In contrast, a high nitrogen dose (400 mg N per seedling) reduced growth performance. The study concluded that combining *Rhizobium* inoculation with a moderate nitrogen level can significantly enhance early seedling growth.

Sumathy and Velu (2025) assessed the diversity and plant growth-promoting potential of rhizobial isolates associated with *Sesbania grandiflora* in the Madurai region. The isolates exhibited multiple growth-promoting traits beyond biological nitrogen fixation. A related glasshouse trial on the same species found that *Rhizobium* inoculation, combined with a lower nitrogen level, increased nitrogen, phosphorus, and potassium concentrations in shoot tissue relative to uninoculated seedlings, indicating enhanced nutrient uptake alongside improved symbiotic performance (Kumar and Dash 2014).

Plant growth-promoting traits of Rhizobia: IAA Production, Phosphate Solubilization, Siderophore Activity, and Stress Tolerance

Surange *et al.*, (1997) found that rhizobial strains isolated from different tree legumes tolerated alkaline conditions up to pH 12.0, while an *Albizia lebbeck* isolate withstood temperatures of 50°C; all tested strains tolerated salt concentrations up to 5.0%, indicating broad abiotic stress tolerance among tree legume rhizobia generally. Such tolerance is agronomically significant given that tree legumes are frequently deployed on marginal or degraded land where soil pH, salinity, and temperature extremes are common constraints. Igiehon *et al.*, (2019) extended the picture under induced drought stress, finding that among five rhizobial strains tested, Ensifer meliloti (R5) produced the highest concentration of 1-aminocyclopropane-1-carboxylate (ACC) deaminase precursor activity under PEG-induced osmotic stress, Darpan (2011) isolated *Rhizobium* from karanj root nodules and evaluated its effects under different nitrogen levels, he found no major pest infestation in inoculated or nitrogen-treated seedlings, whereas control plants showed a minor infestation of red spider mites (*Tetranychus* spp.) at 60 DAT. These findings are consistent with those of Thamer *et al.*, (2011), who

reported that rhizobial nitrogen fixation can enhance plant resistance to herbivores through nitrogen-based defense mechanisms. This suggests that *Rhizobium* inoculation may contribute to increased resistance against mite attack, although further research is needed to confirm this effect.

Phosphate Solubilization

Given that phosphorus deficiency commonly limits plant growth and nitrogen fixation efficiency, phosphate-solubilizing capacity is a key trait in rhizobial PGP screening. Joshi *et al.*, (2023) screened eighteen bacterial isolates from Shisham (*Dalbergia sissoo*) rhizosphere soil for phosphate solubilization alongside other PGP traits, finding solubilization capacity ranging widely from 891.38 $\mu\text{g mL}^{-1}$ in the most effective isolate (L4) to 285.78 $\mu\text{g mL}^{-1}$ in the least effective (T4). Megu *et al.*, (2024) similarly identified phosphate-solubilizing capacity among wild legume rhizobial isolates, with 18 of 84 RPO1-primer-positive isolates testing positive a substantially lower proportion than in Joshi's Shisham-associated isolates, suggesting that phosphate-solubilizing capacity may vary considerably depending on host plant and soil origin. Igiehon *et al.*, (2019) further confirmed phosphate solubilization as a consistent trait across multiple rhizobial species (*Rhizobium*, *Ensifer*), reinforcing it as a near-universal, though variably expressed, PGP trait among tree-legume-associated rhizobia.

Indole Acetic Acid (IAA) Production

IAA production is among the most widely reported PGP traits in rhizobial characterization, typically quantified via the Salkowski colorimetric method. Reported IAA output varies considerably across studies and isolates. Joshi *et al.*, (2023) found IAA production highest in isolates L4, P3, T1, T2, and T4 among their eighteen Shisham rhizosphere isolates, while Megu *et al.*, (2024) reported 38 of 84 wild legume isolates capable of IAA production again indicating that IAA-producing capacity, like phosphate solubilization, is common but not universal among rhizobial isolates. Sumathy and Velu (2025) measured IAA production of 122 mg/L and 104 mg/L in *Sesbania grandiflora*-associated isolates SR1 and SR5 respectively, alongside cytokinin (178–182 mg/L) and gibberellic acid (GA_3 ; 602–688 mg/L) production demonstrating that some tree-legume rhizobial isolates produce a broad spectrum of growth-

promoting phytohormones beyond IAA alone. Igiehon *et al.*, (2019) reported the highest IAA concentrations in *Rhizobium cellulosilyticum* (R3; 22.19–23.16 $\mu\text{g/mL}$ depending on tryptophan concentration), with the lowest in *Ensifer meliloti* (R5), the same strain that showed the strongest ACC deaminase activity under drought stress, illustrating that PGP trait expression often varies independently across mechanisms within a single isolate, reinforcing the value of multi-trait screening panels rather than single-trait selection criteria.

Siderophore and Exopolysaccharide Production

In the study by Joshi *et al.*, (2023), five out of eighteen isolates were found to produce siderophores, with the strongest activity (largest halo zones) observed in isolates L7, L8, T1, and T3. Igiehon *et al.*, (2019) similarly found siderophore production varied by species and growth medium. The same study reported exopolysaccharide (EPS) production, with strain R1 yielding the highest concentrations under both standard and PEG-induced osmotic stress conditions, suggesting EPS production may contribute to desiccation tolerance alongside its established role in root colonization. Traits such as phosphate solubilization,

IAA production, siderophore production, and stress tolerance are usually present only in certain isolates, making it important to screen for multiple traits rather than relying on a single characteristic. Also these traits often depend on the growth medium, host plant, and environmental conditions, meaning an isolate may perform differently under different testing conditions.

Rhizobia as Biofertilizers

The Indian biofertilizer market is projected to grow by 10.1% between 2021 and 2026, driven by expanding organic farming adoption and growing recognition of soil health benefits (Jain *et al.*, 2022), assessed the rhizobial strains obtained from *A. lebbbeck* seedlings collected from six different locations. The isolates III (1) and III (2) recorded the highest nodular biomass and exhibited superior nitrogen-fixing efficiency, with specific nitrogenase activities of 13.73 and 13.59 $\mu\text{mol C}_2\text{H}_2$ reduced h^{-1} and total nitrogenase activities of 11.80 and 11.16 $\mu\text{mol C}_2\text{H}_2$ reduced h^{-1} , respectively. This combination of effective nitrogen fixation and utilization highlights their strong potential as biofertilizer candidates.

Table.1 Taxonomic hierarchy of the genus *Rhizobium* (LPSN, accessed May 2026)

Rank	Name
Domain	Bacteria
Kingdom	<i>Pseudomonadati</i>
Phylum	<i>Pseudomonadota</i>
Class	<i>Alphaproteobacteria</i>
Order	<i>Hyphomicrobiales</i>
Family	<i>Rhizobiaceae</i>
Genus	<i>Rhizobium</i> (Frank, 1889)

Table.2 Tree legume host – bacterium association table

Tree Legume Host	Subfamily	Dominant Genera Reported
<i>Albizia lebbeck</i>	Caesalpinioideae (mimosoid)	<i>Rhizobium</i> , <i>Mesorhizobium</i> , <i>Bradyrhizobium</i>
<i>Leucaena leucocephala</i>	Caesalpinioideae (mimosoid)	<i>Rhizobium</i> , <i>Mesorhizobium</i> ; rarely <i>Bradyrhizobium</i> / <i>Cupriavidus</i>
<i>Vachellia nilotica</i>	Caesalpinioideae (mimosoid)	Both alpha-rhizobia (<i>Rhizobium</i> , <i>Mesorhizobium</i>) and beta-rhizobia
<i>Pongamia pinnata</i> (syn. <i>Millettia pinnata</i>)	Papilionoideae (Faboideae)	<i>Bradyrhizobium</i> and <i>Rhizobium</i> (mixed, region-dependent)

An important observation from the study was that nodular biomass showed a stronger relationship with nitrogen-fixation efficiency than nodule number alone, suggesting that it may serve as a more reliable criterion for selecting effective rhizobial strains. A comparative evaluation of seven nitrogen-fixing tree species (NFTS) showed that *Leucaena leucocephala* hosts the most efficient rhizobial symbionts among the species studied. Its nitrogenase activity per plant was substantially higher than that recorded in *Acacia auriculiformis*, *A. mangium*, *Dalbergia sissoo*, *Albizia lebbeck*, *A. procera*, and *A. catechu*, exceeding them by 280.6%, 336.8%, 1242.3%, 136.2%, 160.3%, and 1993.5%, respectively. These findings are in agreement with the earlier study of Pokhriyal *et al.*, (1987), who reported significantly higher nitrogenase activity in *Leucaena leucocephala* than in *Albizia lebbeck*, *Acacia catechu*, and *Dalbergia sissoo*. The performance of *leucocephala* to form highly active and efficient nodules, highlights its potential for biofertilizer development. Gordon and Wheeler (1978) demonstrated a strong positive relationship between photosynthetic rate, nodule fresh weight, and nitrogenase activity in *Alnus glutinosa*, highlighting the close link between overall plant productivity and biological nitrogen fixation. The outstanding performance of *Leucaena leucocephala* may be partly due to the

presence of highly efficient native rhizobial strains in its root environment. Such strains are of particular interest for biofertilizer development because they can establish more effective symbiotic associations with their host plants. Rosenblueth *et al.*, (2018) reported that rhizobia can also colonize the roots of non-legume plants, even though they do not form nitrogen-fixing nodules in these hosts. In such associations, rhizobia can promote plant growth through mechanisms similar to those used by other plant growth-promoting bacteria (PGPR). Although this falls outside the main focus of tree legume inoculant development, it highlights the potential for rhizobial isolates to be used more broadly in promoting plant growth across different crop species.

In conclusion, Tree legume-associated rhizobia are important components of sustainable agriculture and ecological restoration, contributing to soil fertility, plant growth, and the rehabilitation of degraded lands through biological nitrogen fixation. As highlighted throughout this review, these bacteria exhibit considerable diversity in their taxonomy, host associations, symbiotic effectiveness, and plant growth-promoting traits, which means that the success of rhizobial inoculation depends not only on the presence of compatible rhizobia but also on the careful selection of isolates with desirable

functional characteristics. The studies reviewed emphasizing the need for comprehensive, multi-trait evaluation of isolates. Future research with focus on exploring rhizobial diversity in underrepresented regions, will help improve our understanding of host-microbe interactions better, and validating promising isolates under field conditions. Such efforts will strengthen the development of effective biofertilizers and support broader goals of reducing dependence on chemical fertilizers, improving soil health, and promoting sustainable agroforestry and land restoration practices.

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Author Contributions

Sampi Romin conceived and designed the review; Dr. Diptimayee Dash conducted the literature search and drafted the manuscript and critically; all authors approved the final version and agree to be accountable for all aspects of the work.

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There are no competing interests to declare.

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