



REVIEW ARTICLE

Exploring the potential of beta rhizobium in nitrogen fixation and agricultural sustainability

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Abstract

Some of the earliest discovered nitrogen-fixing symbiotic prokaryotes were the 'Rhizobia,' microbes that associate with legume. A relatively recent group of nitrogen-fixing bacteria, beta-rhizobia plays a significant role in sustainable agriculture. Detailed insights into the relationships between beta-rhizobia and leguminous plants can be found in the phylogeny and taxonomy section on legumes. Here, we provide a discussion of recent literature focusing on the molecular aspects of beta-rhizobia-plant interactions, with potential implications for enhancing nitrogen fixation beyond nodulation processes. Furthermore, we emphasize the importance of coordinating knowledge from other disciplines in to harness these beneficial microbes and advance sustainable crop farming practices. In other words, this review aims to explore the potential of beta-rhizobia and their contributions to sustainable agriculture.

Keywords

Beta-rhizobia; *Paraburkholderia*; nodulation; phylogeny

Introduction

Nitrogen (N) fixation plays a major role in global agricultural and food security (1). It is estimated that 65% of the total N used in agriculture is supplied through N-fixation (2). Nitrogen is essential for sustainable crop production and N-fixation is the primary method of supply (1). The legume-rhizobia symbiosis plays a significant role in sustainable agriculture as it fixes atmospheric nitrogen (N₂) through biological nitrogen fixation (BNF), helps in enhancing soil fertility (3). Similarly, BNF provides an environment-friendly and economically better alternative to chemical fertilizers, promoting sustainable agriculture and maximizing benefits through legume-based crop rotation or intercropping (4).

Rhizobium, a crucial nitrogen-fixing and nodulating bacterium, is of considerable significance in agri-foods owing to its capacity to enhance nutrient availability and stimulate plant growth and development (5). In recent years, the use of rhizobial strains has grown due to advancements in molecular biology that enhance N-fixation and competitive ability (6). The symbiotic relationship between legumes and rhizobia has been a major area of research for more than a century, due to its major impact on sustainable agriculture, soil fertility enhancement and wider ecological benefits (7). Previously, it was thought that the bacteria responsible for nodulation in the majority of legume species were confined to a single genus, *Rhizobium*. However, a breakthrough in bacterial

phylogenetics, achieved by the analysis of 16S ribosomal RNA sequences, changed this understanding, resulting in the classification of rhizobia into various genera (8). Apart from *Rhizobium*, these newly identified groups included *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium* and *Azorhizobium*, all classified under the *Alphaproteobacteria* subclass, generally known as alpha-rhizobia (9).

Certain prokaryotes engage in biological N-fixation, forming symbiotic relationships with plants to fix N (10). Several genera, like *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* can also be placed under a category called 'Rhizobium' or 'rhizobia' (11). Under N-deficient conditions, these plants develop root nodules where rhizobia reside. These nodules provide ideal conditions for the N reduction processes that lead to the conversion of N₂ into ammonia (NH₃) (11).

Beta-rhizobia is a less diverse group within the class *Betaproteobacteria* (5). Beta-rhizobia species, particularly those in the genera *Burkholderia* and *Paraburkholderia*, are vital for N-fixation through their symbiotic associations with legumes (10). These bacteria convert N₂ to NH₃, which plants use for growth. Research has shown that beta-rhizobia regulates the transcription of the nitrogenase gene responsible for this conversion, responding to specific signals from plants or environmental conditions (5). Beyond N₂ fixation, rhizobia, including beta-rhizobia, play diverse ecological roles. They interact extensively with soil microbiomes and plant roots in the rhizosphere (12), competing with other microorganisms to colonize the roots and respond to plant signals (13). Their biocontrol potential against soil-borne plant pathogens involves complex interactions among the plant, pathogen, biocontrol agent and microbial community (14). Mechanisms of biocontrol include antibiosis, competition, parasitism and induced plant resistance. The use of multiple microbial agents, including rhizobia, can enhance biocontrol efficacy compared to single-agent approaches (14).

Currently, beta-rhizobia are classified into three genera; *Cupriavidus*, *Paraburkholderia* and *Trinickia*, all under the family *Burkholderiaceae* (15). The genera *Paraburkholderia* and *Trinickia* were identified through whole-genome analyses of some former *Burkholderia* species. The symbiotic species within *Cupriavidus* was initially recognized as *Ralstonia*. Beta-rhizobia is primarily isolated from root nodules of tropical legumes, such as *Mimosa* species (15), as well as other legumes (16). Notably, beta-rhizobia contributes to N-fixation by regulating bacterial auxin synthesis during symbiosis. For example, the N-fixation activator NifA represses the expression of bacterial auxin synthesis genes, controlling the abundance of auxins like indole acetamide (IAM) and indole acetic acid (IAA) (10). This finding suggests that auxin plays a role in maintaining functional root nodules and enhancing nitrogenase activity.

Although both alpha and beta-rhizobia can fix N₂, they differ in host range and ecological adaptation (17). Alpha-rhizobia forms symbiotic associations with a wide range of leguminous crops; including lucerne, soybean and peas, which are widely used in agriculture worldwide (17, 18). These bacteria contribute to BNF, enhancing soil fertility, minimizing the need for synthetic fertilizers and promoting sustainable

farming (5, 19). In contrast, beta-rhizobia, especially *Burkholderia* and *Cupriavidus* in the *Betaproteobacteria* subclass, are well-suited for acidic or nutrient-poor soils (20). This ability to nodulate less common legumes, particularly in tropical and subtropical regions, makes them valuable for boosting crop yields in marginal or degraded areas (20).

In addition to N-fixation, both alpha- and beta-rhizobia contribute to soil health, plant resilience and disease suppression (5, 19). Alpha-rhizobia increases plant systemic resistance, reducing the need for chemical pesticides (19). Similarly, beta-rhizobia has biocontrol potential, releasing antimicrobial compounds like pyrrolnitrin and pyochelin that inhibit pathogen growth in soil (21, 22). Beta-rhizobia also produces siderophores, which chelate iron from the surrounding environment, limiting nutrient availability for harmful bacteria and other pathogens, thereby inhibiting their growth (23). Furthermore, beta-rhizobia plays a role in phytoremediation, enabling plants to survive and restore contaminated soils by immobilizing heavy metals and degrading harmful chemicals (21). These ecological roles make both groups essential for developing more sustainable agriculture practices, especially where industrial farming faces challenges.

Overall, beta-rhizobia, along with alpha-rhizobia, are vital components of sustainable agriculture, particularly in developing countries, as they enable the conversion of N₂ into a bioavailable form, reducing the need for synthetic nitrogen fertilizer (19, 24). The primary differences between alpha and beta-rhizobia are demonstrated in Table 1.

Taxonomy and Phylogeny of beta-rhizobium

Recent literature emphasizes the importance of mutualistic interactions between legume plants and rhizobia in N acquisition within terrestrial environments (25). Most beta-rhizobia belong to the class *Betaproteobacteria*, under the domain *Pseudomonadaota*, also known as *Proteobacteria*. These beta-rhizobia differ from the previously known alpha-rhizobia belonging to the class *Alphaproteobacteria* (26). Recent research has identified and characterized novel root nodule bacteria isolated from native legumes, particularly those from the genus *Paraburkholderia*, which were previously classified under *Burkholderia* (26, 27).

Phylogenetic investigations of beta-rhizobia with other species of the *Rhizobium* genus affirm that certain recently identified rhizobia are classified within the beta-rhizobia group of the *Paraburkholderia* genus. Notably, these bacteria have been found to interact symbiotically with tree species such as *Vachellia karroo* in South Africa (26). Furthermore, genetic studies have highlighted the significant diversity of rhizobia forming nodules with different leguminous plants, demonstrating that the beta-rhizobia group encompasses numerous species (10) (25). A phylogenetic tree depicting the evolutionary relation between various species under the rhizobia species of *Betaproteobacteria* is illustrated in Fig. 1.

The taxonomy and phylogeny of beta-rhizobia, particularly those within the genus *Paraburkholderia* are organized to enhance understanding of their legume symbiosis and N-fixing activity in terrestrial environments (28). For the past two decades, the focus has shifted from 16S rRNA

Table 1. Characteristic difference between alpha and beta-rhizobia

Criteria	Alpha-rhizobia	Beta-rhizobia	Reference
Taxonomic Classification	Belong to the subclass <i>Alphaproteobacteria</i> . Includes genera like <i>Rhizobium</i> , <i>Bradyrhizobium</i> , <i>Sinorhizobium</i> , <i>Mesorhizobium</i> , <i>Allorhizobium</i>	Belong to the subclass <i>Betaproteobacteria</i> . Notable genera include <i>Burkholderia</i> and <i>Cupriavidus</i>	(18)
Nodulating Host Range	Primarily associated with temperate and tropical legumes, including common crops like peas, beans and soybeans	Nodulate both leguminous and non-leguminous plants, particularly adapted to tropical legumes and some non-traditional hosts.	(18)
Geographical Distribution	Widely distributed across temperate and tropical regions	Typically found in tropical and subtropical regions, especially in acidic or nutrient-poor soils	(18)
Symbiosis Mechanism	Use lipo-chitooligosaccharide (LCO) signals known as Nod factors for initiating nodulation.	Also, use Nod factors but can form a more flexible symbiosis, especially in challenging environments.	(18)
Stress Tolerance	Tolerant to certain environmental stresses but generally less adaptable to extreme conditions like acidity or heavy metals.	Better suited for extreme environments such as high acidity, heavy metals, and saline soils. The use of exopolysaccharides (EPS) and ACC deaminase improves tolerance in saline soils.	(18)
Ethylene Lowering	Some alpha-rhizobia also produce ACC deaminase, but this is more studied in beta-rhizobia.	Known for producing ACC deaminase, lowering plant ethylene levels to enhance nodulation, especially under stress conditions	(18)
Horizontal Gene Transfer (HGT)	HGT is a known mechanism in alpha-rhizobia but is less common for major adaptive traits.	More dynamic in HGT, contributing to greater adaptability and the acquisition of nodulation genes from other bacteria	(18)
Applications in Agriculture	Widely used in biofertilizers for legumes like soybeans, lentils, and peanuts.	Emerging potential in agriculture, especially for crops in nutrient-poor soils or challenging climates	(18)

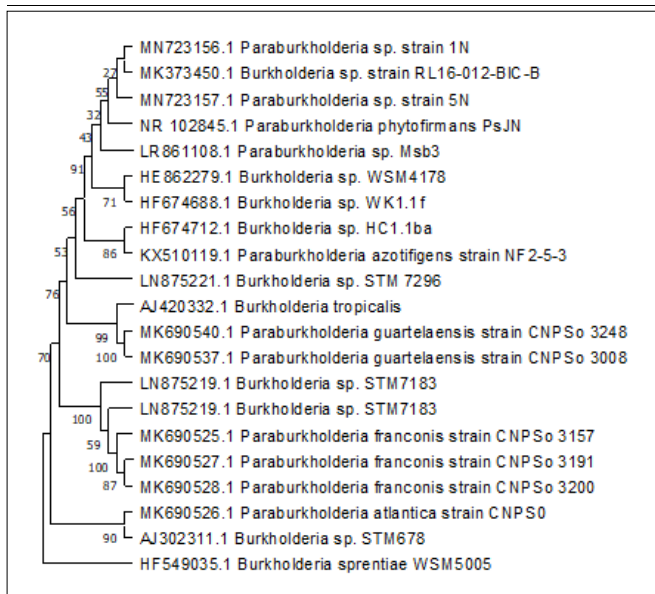


Fig.1. 16S rDNA tree showing phylogenetic positions of legume nodulating *Paraburkholderia* and *Burkholderia* species within the *Betaproteobacteria*. (Sequence retrieved from NCBI <https://www.ncbi.nlm.nih.gov/> and phylogenetic tree created using MEGA 11 software.)

sequencing and analysis toward genotaxonomy and average nucleotide identity (ANI) (28). *Paraburkholderia* is noted for its diverse patterns of symbiotic genes and nodulation efficiency, which complicate its classification based on genome-scale data. Core genes, including *dnaK*, *gyrB*, *rpoB*, *glnA*, *glyII*, *atpD* and others, have been used to construct phylogenetic trees, though some genes show inconsistencies in delineating taxonomic boundaries (29).

Recently, novel phylogenetic and taxonomic markers, such as *SMc00019*, *truA* and *thrA*, have been identified to assist in constructing more robust phylogenetic trees for rhizobia (29). This field is evolving rapidly, with emerging techniques

and findings likely to refine our understanding of beta-rhizobia taxonomy and phylogeny further. Studies on beta-rhizobium strain diversity and distribution have been particularly focused on locations like the Fynbos biome in South Africa (30,31). Research shows that beta-rhizobia nodulates a variety of legume species, such as *Podalyria calyptata*, *Vachellia karroo* and *Vigna minima*, indicating a wide range of host plants for these bacteria (30-32).

Considerable genetic diversity has been observed among beta-rhizobia strains, with no clear correlation between genetic and geographic distances between populations. High rates of dispersal and colonization within soils have also been documented (31). Genetic characterization of these strains has employed various molecular techniques to establish their diversity at species and strain levels accurately (30, 32). Symbiotic associations between beta-rhizobia and various legume species demonstrate both specificity and promiscuity, with tribes like *Crotalariaeae* and *Indigofereae* able to nodulate with both alpha- and beta-rhizobia (33). Further research is needed to fully understand the evolutionary relationships, distribution patterns and ecological roles of beta-rhizobium strains across different environments.

The interactions between symbiotic partners can influence one another, leading to the gain or loss of symbiotic genes through horizontal gene transfer (HGT). Some rhizobial genera, like *Bradyrhizobium*, can nodulate legume subfamilies (*Mimosoideae*, *Papilionoideae* and *Caesalpinioideae*), while others, such as *Neorhizobium galgae* and *Rhizobium leguminosarum*, exhibit a narrow host range (34). The diversity of rhizobia provides a pool of symbiotic bacteria from which compatible legumes can select; this selection may involve a single strain or multiple bacterial cells that adapt to the

heterogeneity of each plant withstand varying environmental conditions and withstand varying conditions over time and space (34).

Additionally, the two symbiotic partners start to influence one another. When combined, this may cause symbiosis genes to be gained or lost through HGT. Some rhizobial genera, like *Bradyrhizobium*, are capable of nodulating legumes across all three legume subfamilies (*Mimosoideae*, *Papilionoideae* and *Caesalpinioideae*), while others such as *Neorhizobium galegae* and *R. leguminosarum* have a very narrow host range (34).

Horizontal gene transfer is significant in the adaptability and evolution of beta-rhizobia, including *Burkholderia* and *Cupriavidus* (35, 36). Horizontal gene transfer, facilitated by conjugation, transformation and transduction, allows beta-rhizobia to obtain genes from other species, hence granting them symbiotic capabilities and environmental resilience. These genes allow beta-rhizobia to establish N-fixing nodules on new hosts (36, 37). Transformation involves the uptake of free DNA from the environment, which provides traits such as antibiotic resistance and stress tolerance. Mechanisms like these enable an adaptive process to several environments, most importantly, poor or extreme soils in an efficient manner (37).

Horizontal gene transfer also helps in expanding the host range of beta-rhizobia, enabling them to form symbiotic relationships with legume species that they previously could not. This genetic flexibility is beneficial in the tropics and subtropics, where beta-rhizobia interacts with different legume hosts (5). In addition, the acquisition of genes involved in antimicrobial compound biosynthesis further improves the biocontrol potential of beta-rhizobia, allowing them to act as natural biopesticides and to control soil pathogens effectively. Overall, HGT encourages the evolution of beta-rhizobia by enabling adaptation to environmental stresses and expanding their ecological niches, thus enhancing their agricultural applications as biofertilizers and biocontrol agents (37, 38).

The identification of nodulating bacteria beyond the conventional rhizobia from the *Alphaproteobacteria* leads to significant research into the evolutionary relationships and classification of these newly discovered nodulating strains within the *Betaproteobacteria* group (39). This effort led to the characterization of two novel nodulating strains, named *Burkholderia phymatum* STM815 and *Burkholderia tuberum* STM678 (39).

Novel strains of beta-rhizobia, particularly those from the genus *Burkholderia* (recently renamed *Paraburkholderia*), have been identified in root nodules of various indigenous as well as invasive legumes worldwide, particularly in South America and Southeast Asia (26, 27). These beta-rhizobia exhibit distinct phylogenetic and taxonomical differences from traditional "alpha-rhizobia" such as *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium* (27). The ability of beta-rhizobia to establish nodules with legumes challenges the long-standing concept of host specificity in legume-rhizobia interactions (27). Some legumes, like *Vachellia karroo* in South Africa, are promiscuous hosts, able to create efficient nodules with both alpha and beta-rhizobia (26).

Environmental factors influencing growth and symbiotic interactions

Various environmental conditions influence the symbiotic relationship between legumes and beta-rhizobia. Stress factors that can impact this symbiosis and its effectiveness include heavy metals, dehydration, salt, fluctuations, temperature, pH variations and the presence of microbial pathogens (33). For instance, salt stress can disrupt rhizobial symbiosis formation in legumes when NaCl concentrations are as low as 40 mM, reducing the incidence of rhizobial infections in a variety of legumes, including peas, beans and soybeans (18). The development of nod factors is impacted by the accumulation of harmful ions in saline soil, which is connected with low-quality flavonoids in legume root exudates (RE) (18). Additionally, the non-N-fixing microbial community significantly influences the legume-rhizobia symbiosis (27).

To achieve the full potential of this symbiotic interaction, the host plant must maintain healthy growth and withstand harsh environmental conditions (18). Physiological adaptations in plants are crucial for sustaining their symbiotic relationship with rhizobia under salt stress, drought and heavy metal exposure. These responses include an increase in the activity of antioxidant enzymes, primarily superoxide dismutase and catalase, which neutralize reactive oxygen species (ROS) generated under stress (18).

Plants also regulate osmotic balance by accumulating osmoprotectants, such as proline and glycine betaine, which help maintain cellular integrity and protect the rhizobia within the nodules (40). Because this osmotic adjustment is connected to changes in root exudate composition, nodulation signals like flavonoids continue to function effectively despite stress (41). Hormonal regulation, including an increase in abscisic acid, also plays a vital role in maintaining nodule formation and N-fixation during stress (27).

This protective mechanism shields plant cells and N-fixing bacteria within the nodules from oxidative damage, thereby sustaining symbiosis under stress conditions (40). The complex signal exchange between the symbiont and the host is now recognized as essential for the legume-rhizobia interaction. Rhizobia are also vulnerable to challenges such as oxidative stress, osmotic stress, low pH and growth-inhibiting plant peptides (41). Both plant and bacterial partners rely on stress tolerance mechanisms to sustain their symbiotic relationship (41).

Cellular structure and morphology

The cellular structure and morphology of beta-rhizobia, with special reference to the genus *Cupriavidus* which was formerly known as *Rhizobium*, are closely linked to their ability to form N-fixing root nodules with leguminous plants (42). When describing the cellular aspects of beta-rhizobia, attention is often given to their location within infection threads and bacteroids in the nodules (43). Infection threads are tube-like outgrowths of host plant cells that enclose the rhizobia. They arise from the root hairs and penetrate the adjoining tissue (44). The conditions inside infection threads physically confine the rhizobia within an exopolysaccharide (EPS) layer and the plant cell cytoplasm. The EPS layer in rhizobia serves several important functions beyond merely confining them in the

infection thread. It helps rhizobia evade plant defense mechanisms, protecting them from reactive oxygen species (ROS) and antimicrobial peptides (45). Additionally, the EPS layer protects rhizobia from osmotic and environmental stress within the nodule, promoting symbiotic efficiency by creating a favorable environment for N-fixation (40). Exopolysaccharide-deficient mutants typically show reduced infection and nodulation success, highlighting its critical role in the rhizobia-legume symbiosis. Furthermore, EPS enhances bacterial adhesion to plant root hairs, an essential step in initiating infection. EPS is crucial for proper infection thread formation, as mutants lacking EPS production often exhibit impaired nodulation (46). Infection threads facilitate the transport of rhizobia throughout plant tissues, providing nourishment to the rhizobia while shielding them from early exposure to plant defense mechanisms (44).

Once inside the plant cells, rhizobia differentiate into bacteroids, which are specialized for N-fixation. Bacteroids no longer reproduce but instead focus on converting N₂ into ammonia, which the plants use. The symbiosome, consisting of a plant-derived membrane surrounding the bacteroid, resembles an organelle-like structure, indicating a co-evolved system (47).

Overall, the cellular structure of beta-rhizobia is integral to efficient N-fixation and the establishment of mutualistic relationships with legume plants (47).

Infection and nodulation

Rhizobia can infect plants through two distinct pathways. The less common entry point is through cracks, in the epidermal or cortical cells (48). More commonly, rhizobia infects plants *via* root hairs, a process triggered by nodulation factors (NFs) (48). Plant flavonoids activate the transcriptional regulator NodD, which belongs to the LysR family of transcriptional activators. NodD, in turn, regulates the expression of *nod* genes, which encode the structural components responsible for NFs (49). These factors act as morphogenic signal molecules, inducing early responses like calcium spiking, root hair deformation and cortical cell division, which eventually result in the formation of functional nodules (10).

Nod factors are composed of chitin oligomers with two to six β -(1,4)-linked N-acetyl-D-glucosaminyl residues, modified at their non-reducing ends. Two groups of nod genes have been identified: "species-specific" genes and "common" genes (*nodA*, *nodB*, *nodC*, *nodI* and *nodJ*), which are shared by all rhizobial species that communicate with their hosts through the NF. The "common" genes, *nodA*, *nodB* and *nodC*, encode the acyl transferases, deacetylases and N-acetylglucosaminyl transferases, respectively, that make up the core structure of NFs. Additionally, the *nodIJ* genes, present in all rhizobia, encode an ABC transporter essential for NF secretion.

The species-specific genes (*noe*, *nol* and additional *nod* genes) encode enzymes that introduce various modifications to the NF core, such as N-methyl groups by *nodS*, sulfate groups by *nodH*, or O-carbamoyl groups by *nodU* (50). These modifications result in a diverse range of NFs, enabling rhizobia to adapt to new host plants. This ability to diversify NFs highlights the evolutionary flexibility of rhizobia in establishing symbiosis with a variety of legumes (50).

Genetic modification within the rhizobial group enables structural diversification of nodulation factors involved; these factors play a crucial role in the specificity of interactions between various legume species (49). Nod factors, belonging to the broad category of LCOs, serve as signaling molecules between rhizobia and host plants, triggering nodule formation. Variation in the chemical structure of these molecules—such as acetylation, sulfation, or glycosylation, or the chain length of the acyl group—plays a critical role in determining host specificity and the establishment of successful symbiosis (23). These structural modifications allow rhizobia to adapt to a wide range of legume species and potentially colonize new hosts by fine-tuning the Nod factor signaling pathway (51).

For instance, the addition of sulfate or acetate groups to Nod factor molecules can significantly influence recognition by the host plant. Sulfated groups are often essential for symbiosis with specific legumes like alfalfa (*Medicago sativa*), where receptor kinases on the plant root specifically recognize these modifications. In contrast, non-sulfated Nod factors may enable rhizobia to form symbiotic relationships with legumes that do not require sulfation, thereby expanding the host range. This structural flexibility highlights the evolutionary adaptations of rhizobia to varied ecological niches, facilitating interactions with multiple legumes across variable environmental conditions (49).

Additionally, structural changes in Nod factors influence the efficiency with which rhizobia invades the root hair cells of various legume species. Compatibility between rhizobia and host plant root receptors, for example, is influenced by the variations in the length and saturation of the fatty acyl chain (52). Certain rhizobia adapt to host plants with root receptors that specifically recognize long-chain Nod factors, while others might evolve to interact with legumes that require shorter-chain variants. These changes can optimize the rhizobia's ability to trigger infection thread formation, ensuring efficient nodule development and N-fixation across a range of legume species (11).

While alpha and beta-rhizobia share similar nod core genes, they differ in the presence of species-specific genes. Root exudates (RE) induce beta-rhizobial nod genes, as demonstrated by *Mimosa pudica* REs in *Paraburkholderia phymatum* and *Cupriavidus taiwanensis* (50). In *Paraburkholderia* species that nodulate mimosoid legumes, the *nodBCIJHASU* operon typically contains a single copy of each nod gene (50). However, in *Paraburkholderia* species nodulating papilionoid legumes, genes such as *nodB* and *nodC* are duplicated, with *nif* genes and transposases separating the *nodUSDABC* cluster from *nodIJ*. These species also appear to contain *noI* and *nodH* genes, suggesting their Nod factors (NFs) possess carbamoyl groups instead of being sulfated (50). The presence of *nodSU* in both *Paraburkholderia* groups suggests the addition of methyl and carbamoyl groups to the NF core structure (10).

The symbiosis between rhizobia and legumes requires initial recognition and interaction between free-living bacteria and a plant host before rhizobia can nodulate the legume and perform BNF within root nodules (47). Specific signaling molecules facilitate this recognition, including flavonoids and

lectins produced by the host plant, as well as NFs such as EPS or lipochitooligosaccharides (LCOs) produced by rhizobia. Subsequently, variations in gene expression and metabolism in both partners are essential for nodule organogenesis, rhizobial entry into the plant and eventual rhizobia accommodation within the nodule.

Since beta-rhizobia was only recently identified, the molecular pathways underlying their symbiosis with legumes remain less understood (10). However, recent studies analyzing the genetic networks employed by N-fixing *Paraburkholderia* to interact with their plant hosts have unveiled key insights into these mechanisms (53). Recent studies examining the genetic networks that *Paraburkholderia* use to interact with their plant hosts have unveiled important insights into how these bacteria establish nitrogen-fixing symbiosis. These studies highlight the distinctiveness of beta-rhizobia compared to alpha-rhizobia in terms of their genetic makeup and interaction mechanisms. One of the major findings is the identification of novel nodulation genes in *Paraburkholderia*, which allow them to form symbiotic relationships with a broader range of legumes, including non-traditional host species (53).

Moreover, beta-rhizobia produce unique NFs and EPS, which facilitate nodulation under harsh environmental conditions, such as acidic or nutrient-deficient soils. These adaptations allow *Paraburkholderia* to colonize diverse environments and offer potential for enhancing agricultural sustainability through improved N-fixation, particularly in challenging agricultural landscapes (53, 54). These findings represent the cutting edge of research in rhizobial-legume symbiosis, offering new avenues for leveraging beta-rhizobia in sustainable farming practices.

Rhizobia forms microcolonies or biofilms on plant's roots to colonize them. Biofilm formation by rhizobia is a complex, multi-step process that begins with the attachment of rhizobial cells to the surface of plant roots. Initially, rhizobia uses surface proteins, EPS and LCOs to adhere to root hairs (55). Once attached, rhizobia multiply and secrete extracellular polymeric substances, forming a protective matrix around the colony. This matrix helps the bacteria to attach to the root surface and shields them from environmental stress. The biofilm structure also facilitates communication and signal exchange among bacterial cells, enhancing their ability to colonize roots and form infection threads (ITs), which eventually lead to nodule formation and N-fixation occurs (55).

Rhizobial cells travel along ITs within the curled root hairs towards the nodule primordia, where they are released and differentiate into N₂-fixing bacteroids (Fig. 2). The biofilm on the root primarily consists of EPS. After binding to EPS protein receptor 3 (EPR3) of the host, these chemicals deactivate the defense signaling pathway. The function of EPS in beta-rhizobia in initiating root hair curling and IT development has not yet been studied.

The *bceN* gene encodes a protein with GDP-D-mannose 4, 6-dehydratase enzyme activity. It was discovered recently that *P. phymatum* STM815T forms the polysaccharide cepacian. This polysaccharide has been demonstrated to be important in the initial stages of developing a symbiotic relationship with the common bean. It is not the same as the normal EPS found in alpha rhizobia, which are encoded by the *wge/wga* and *exo/exs* gene clusters, both of which are missing from the genomes of *Paraburkholderia* strains.

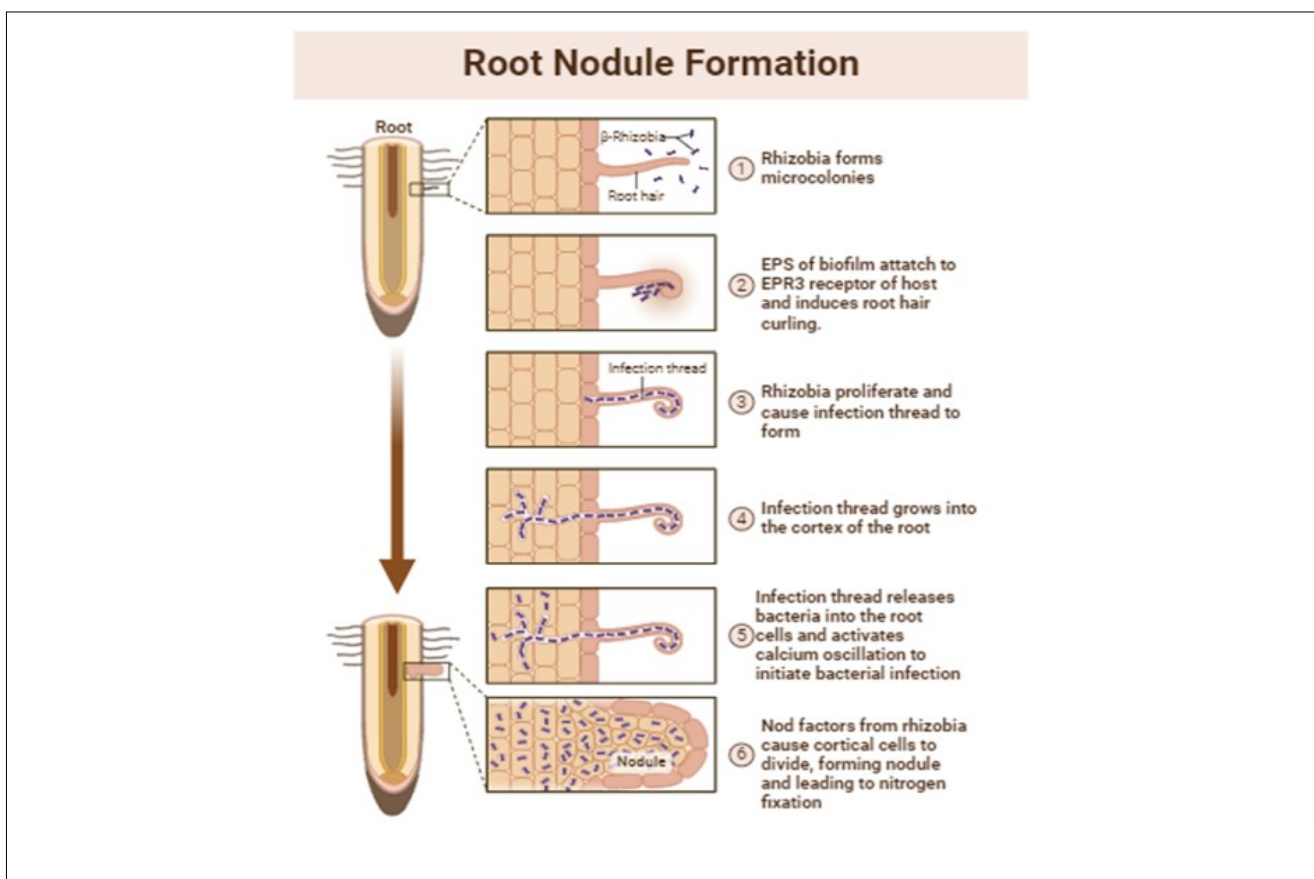


Fig. 2: Series of events during nodulation by beta-rhizobia. The figure is created using BioRender tool. (<https://www.biorender.com/>)

Rhizobia can enhance nodulation by using the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase to lower ethylene levels in the plant root nodules. The bacterial gene *acdS* encodes ACC deaminase, which breaks down ACC, the immediate precursor of ethylene, into ammonium and α -ketobutyrate. Studies have shown that *Mimosa pudica* root exudates (RE) increase the expression of *acdS* and the entire RTX coding operon in *Paraburkholderia phymatum* STM815T. The presence of both ethylene-lowering pathways in a strain could significantly enhance its nodulation capacity.

These ethylene-lowering mechanisms are particularly prevalent in *Bradyrhizobium* and *Paraburkholderia* strains, highlighting their evolutionary importance in symbiotic processes. ACC deaminase, which cleaves the ethylene precursor ACC, is widely distributed among rhizobial groups and is thought to have been acquired through horizontal gene transfer. Co-inoculation with ACC deaminase-producing bacteria and rhizobia has demonstrated potential in promoting nodulation (56).

Understanding these strategies for ethylene modulation could pave the way for developing innovative approaches to enhance N-fixation in agriculture, contributing to sustainable farming practices (57).

Signaling mechanisms between Beta-Rhizobium and host plants

Although research on the relationship between legumes and beta-rhizobia is less extensive than that on alpha-rhizobia, some data is still available. While beta-rhizobia are not as commonly associated with N-fixation as alpha-rhizobia, they offer unique opportunities to further our understanding of this essential symbiosis (53). A coordinated exchange of signals between the symbionts and the host plant is one of the complex signaling processes that result in effective symbiosis between legumes and rhizobia (53). The formation of legume-rhizobia symbiosis is regulated by primary signaling events that occur sequentially. Rhizobia are attracted to the plant root system by flavonoids released by the plant, initiating a series of coordinated actions leading to nodulation (54).

The plant then recognizes the Nod factors produced by the rhizobia, which triggers a chain of events culminating in nodule development (54). All nodulating rhizobia have the common nod genes, *nodABC* and the regulatory gene *nodD*, which synthesize the lipochitooligosaccharide backbone. These genes are somewhat interchangeable among rhizobial species. Host-specific nod genes, however, are unique to a given host species and are not shared by other rhizobial strains; they distinguish between the different substituents on the Nod factor backbone (53).

Role in sustainable agriculture and soil fertility

Beta-rhizobia, specifically members of the genus *Rhizobium*, play a crucial role in sustainable agriculture along with soil fertility enhancement (4). They are primarily recognized for their capability to form symbiotic relations with leguminous plants, fixing N_2 into forms usable by plants (58). This N-fixation reduces reliance on chemical fertilizers and helps maintain soil fertility. Additionally, *Rhizobium* species contribute to plant growth through several mechanisms, such as the solubilization of mineral nutrients like phosphorus,

potassium, calcium and sulfur, as well as the production of plant growth regulators and biocontrol agents against plant diseases (4, 58).

Rhizobia improves soil health and productivity, particularly in organic farming systems and demonstrates great promise for enhancing N-fixation in crops beyond just legumes (4). Furthermore, rhizobia inoculants provide a cost-effective and environmentally friendly alternative to chemical fertilizers, offering a high return on investment (4).

Furthermore, *Rhizobium* species exhibit plant growth-promoting characteristics when interacting with non-legume plants, though N-fixation in these cases is limited (58). The effectiveness of *Rhizobium* in enhancing soil fertility and sustainable crop production is influenced by factors such as strain selection, legume genotype, management practices and environmental conditions (59).

In this mutualistic relationship, rhizobia biologically fixes N_2 by converting it into a plant-usable form, enriching soil nutrient reserves and promoting plant growth and development (59). For instance, rhizobia synthesizes compounds like lumichrome, riboflavin, gibberellin, cytokinin, and indole-3-acetic acid, which enhance plant growth (60, 61). In addition to N-fixation, these compounds improve nutrient availability in the root zone, solubilize and release phosphorus in the soil, enhance plant iron nutrition, chelate heavy metals and act as antimicrobial agents against pathogens. These multifunctional roles directly contribute to plant growth and yield enhancement (60, 61).

Members of the genus *Paraburkholderia*, known for their plant growth-promoting abilities, significantly enhance crop yields while reducing reliance on chemical inputs (62). These bacteria contribute to N-fixation, phytohormone production and regulation, and the neutralization of harmful substances (63). A newly isolated species *Paraburkholderia* strain Msb3, has been shown to enhance the growth of tomato seeds by about 40% of total plant biomass (63). However, some strains of bacteria can be opportunistic pathogens, raising concerns about their widespread agricultural application (63). As plant growth-promoting rhizobacteria (PGPR), *Paraburkholderia* species influence crop yields both directly and indirectly by modulating the plant hormonal system, inducing pathogen resistance and solubilizing nutrients (64). Nevertheless, further research is needed to optimize the use of these beneficial bacteria under field conditions (62).

The mutual interaction between rhizobia and legumes not only fixes N but also influences root system architecture and overall plant growth (65). Under N-limited conditions, this symbiosis significantly influences root system architecture and resource allocation, leading to improved nutrient acquisition and redistribution within the plant (65). Overall, the rhizobia-legume mutualism represents a promising avenue for increasing crop productivity by enhancing nutrient availability, stimulating growth and supporting symbiotic N-fixation (66).

Environmental impacts and ecological significance

This group of N-fixing bacteria, known as rhizobia, has multidimensional uses in environmental bioremediation and

agriculture practices. Here are some key points regarding the environmental impacts and ecological significance of Rhizobium:

Beta-rhizobia plays a crucial role not only in N-fixation but also in other ecologically significant processes such as bioremediation and carbon sequestration (67). These bacteria have the remarkable ability to degrade organic pollutants, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and other toxic compounds in contaminated soils. By facilitating the degradation of these contaminants, rhizobia aids in soil remediation, improving soil health and mitigating environmental risks (68). Additionally, bioremediation through rhizobia can enhance carbon sequestration within the soil, as the degradation of organic pollutants often leads to the stabilization of carbon, preventing its release into the atmosphere as greenhouse gases. This capacity to mediate carbon dynamics positions rhizobia as critical agent in climate change mitigation efforts.

Beta-rhizobia also influences the structure and function of microbial communities in the soil. They influence the population of microbial degraders, either promoting beneficial interactions or suppressing harmful organisms (69). This interaction can positively impact ecosystem restoration by regulating microbial dynamics, and helping to rebuild healthy, functioning soil ecosystems. Ecologically, rhizobium-legume symbioses are of great importance as they fix N_2 , reducing the need for synthetic N fertilizers, which are associated with greenhouse gas emissions and soil degradation (69). This natural process supports sustainable agriculture by enhancing plant growth and promoting soil fertility.

Moreover, beta-rhizobia impacts plant community diversity and structure by influencing the composition of plant species in ecosystems. By improving nutrient availability, they indirectly support a diverse range of plants, playing a role in maintaining the ecological balance and resilience of natural systems. Additionally, Rhizobia is highly adaptive to a range of environmental conditions, including temperature and pH variations, and the hazardous metal ions present (6). This adaptability ensures their survival and effectiveness in diverse environments, from acidic soils to areas contaminated with heavy metals, making them versatile tools for ecological restoration and sustainable agriculture.

Therefore, while rhizobia bacteria are involved in environment cleanup through bioremediation, the members of this group are useful in the practises of modern sustainable agriculture practices. They help in N-fixation and plant growth while dealing with different parts of different ecosystems.

Reclassification *Burkholderia* to *Paraburkholderia*

The genus *Burkholderia* has been proposed to be divided into two genera based on phylogenetic clustering: the genus *Burkholderia*, which retains its name and is primarily composed of plant and animal diseases and the genus *Paraburkholderia*, which includes environmental bacteria (70). According to the modified genus description, only two of the sixteen distinct species of the genus *Burkholderia* that were published between the effective as well as valid publications of the genus name *Paraburkholderia* could be classified as members of this genus (70).

Based on phylogenetic clustering, it has been suggested to bifurcate the genus *Burkholderia* into two genera: *Paraburkholderia*, which includes environmental bacteria and *Burkholderia*, which will continue to represent the group that mostly consists of plant and animal diseases. To form a separate clade containing 12 species from both genera, it was proposed to move 11 species to *Paraburkholderia* and establish a new genus, *Caballeronia*. Among the redefined species are *Paraburkholderia ginsengiterrae* and *Paraburkholderia dipogonis* (70).

The genus description of *Paraburkholderia* indicates a distinctive trait: none of its species are associated with humans (70). All fourteen of the species studied were isolated from non-human or non-mammalian source materials. In strains from various soil types, ten distinct species of bacteria were found. Two of these species are legume-nodulating bacteria, one was isolated from marine sediment collected in a hydrothermally rich area abundant in arsenic and one is an amino-weathering bacterium isolated from rock surfaces. The diversity of isolation sources highlights the adaptability of these bacteria, allowing them to thrive in a wide range of ecological niches (70).

Conclusion

The increasing discovery of root-nodulating *Betaproteobacteria*, particularly from the *Paraburkholderia* genus, marks a significant shift in our understanding of legume symbiosis. While beta-rhizobia, such as *Paraburkholderia*, show potential for BNF, they have certain limitations when compared to alpha-rhizobia (like *Rhizobium* and *Bradyrhizobium*) (62). First, the symbiotic relation between beta-rhizobia and legumes is often less specific and efficient, in terms of N-fixation rates, which can depend on environmental conditions (7). Beta-rhizobia may not form stable or highly effective symbiotic relationships with a broad range of legume species, limiting their general applications in diverse agricultural fields (7, 10). Additionally, environmental stress such as soil acidity, salinity and drought may affect the survival and efficiency of beta-rhizobia more than alpha-rhizobia, which have evolved specialized adaptations for harsh conditions in certain ecosystems (7). Moreover, there is a lack of extensive research and field data on the long-term effectiveness of beta-rhizobia in real-world farming systems, creating uncertainty about their large-scale viability (71).

These bacteria have been found to form nodules and fix N in collaboration with various native legumes that thrive in acidic and nutrient-deficient soils (43, 72-74). The long-held belief that only rhizobia, or bacteria belonging to the *Alphaproteobacteria* family, are capable of nodulating legumes has been altered by the numerous discoveries that beta-rhizobia can nodulate legumes. However, the understanding of how NF functions as the primary molecular signal determining host specificity between legumes and rhizobia remains incomplete, hindering deeper insights into the mechanisms underlying legume-rhizobium host specificity. The identification of diverse novel species of *Paraburkholderia* (beta-rhizobia) within root nodules and the emergence of new rhizobia present a major microbiological challenge for the future domestication of legumes to improve sustainable agricultural practices (71).

Additionally, it has been suggested that nodulation by the recently emerged beta-rhizobia of the *Burkholderia* genus (now classified as *Paraburkholderia*) appears to be more closely linked to a physical niche, such as sandy, acidic soils, rather than adhering to the traditional concept of legume host specificity for nodulation within a specific legume lineage. The mechanism underlying legume host specificity in the nodulation process is still debated, as in the last 20 years, several unique species of rhizobia that nodulate more than one legume host have been discovered. Furthermore, several native legumes, such as those in the Papilionoid group found in South Africa, are nodulated by multiple rhizobium species, including both alpha- and beta-rhizobia. Understanding the mechanisms underlying legume-rhizobium host specificity is essential and it is crucial to determine whether Nod factors serve as the primary molecular signals responsible for legume-rhizobium host specificity. Rhizobiologists are now concerned (71) that the discovery of several new species of *Paraburkholderia* (beta-rhizobia) from root nodules and the emergence of new rhizobia may pose a microbiological challenge for the domestication of legumes in the future to promote sustainable agriculture (27).

Despite the limitations, beta-rhizobia holds significant promise for sustainable agriculture due to its potential for niche adaptation and the ability to nodulate legumes that alpha-rhizobia may not interact with. In future agricultural systems, beta-rhizobia could be tailored to specific soil conditions or crop systems where alpha-rhizobia is less efficient. Genetic engineering and microbial community management may also enhance the symbiotic efficiency and stress tolerance of beta-rhizobia, broadening their applicability (7). Additionally, *Paraburkholderia* species exhibit traits such as phosphate solubilization and production of plant growth-promoting substances, which can further enhance their role in promoting plant health and resilience (5). Future research should focus on developing bioinoculants that combine beta-rhizobia with other beneficial microbes to create more robust and sustainable soil ecosystems, potentially leading to reduced reliance on synthetic fertilizers.

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