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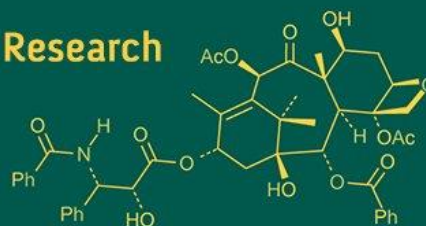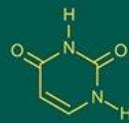
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The emerging importance of nodule-associated bacteria in sustainable agriculture

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Abstract
Legume root nodules host a diverse community of microorganisms that play essential roles in biological nitrogen fixation and plant health. While classical rhizobia are well-known for their ability to convert atmospheric nitrogen into plant-available forms via nitrogenase, increasing attention is now directed toward nodule-associated bacteria (NAB), a group of non-rhizobial endophytes once dismissed as contaminants. NAB contribute to plant growth through multiple mechanisms, including enhanced nutrient acquisition, phytohormone production, modulation of plant defenses, and increased tolerance to drought, salinity, and heavy metal stress. Their interactions with rhizobia can further improve nodulation efficiency and nitrogen-fixing potential. Understanding the cross-inoculation capabilities, specificity, and metabolic functions of both rhizobia and NAB- supported by metagenomics, isotopic and non-isotopic nitrogen-fixation assays, and classical techniques such as ARA and ^{15}N dilution- is crucial for optimizing legume productivity. Collectively, these insights highlight the potential of rhizobia- NAB consortia as powerful bioinoculants for sustainable agriculture.

Keywords: Root nodules, diazotrophy, symbiotic efficiency, plant–microbe interaction, non-rhizobial endophytes

Introduction
Legumes hold a central place in sustainable agriculture because of their remarkable ability to form root nodules- specialized organs where atmospheric nitrogen is biologically fixed into plant-usable ammoniacal nitrogen through the action of the enzyme nitrogenase. While nodulation is widespread in the Papilionoideae, frequent in Mimosoideae, and rare in Caesalpinioideae, these patterns also offer evolutionary insights into the development of symbiotic nitrogen fixation (Allen and Allen, 1981) [2]. Historically, all microorganisms recovered from root nodules were collectively referred to as root nodule bacteria, a term that blurred the distinction between classical nitrogen-fixing rhizobia and other cohabiting microbes (Sturz *et al.,* 1997) [62]. Recent advances have clarified this complexity, recognizing these additional microbial inhabitants as non-rhizobial endophytes or nodule-associated bacteria (NAB) (Rajendran *et al.,* 2012) [49]. Increasing evidence highlights that NAB play crucial roles far beyond mere co-occurrence within nodules. They enhance plant growth by improving nutrient acquisition, producing phytohormones, modulating immunity, and assisting plants in tolerating abiotic stresses such as drought, salinity, and heavy metal toxicity. Many nonpathogenic NAB also act synergistically with rhizobia, potentially strengthening nitrogen fixation efficiency and overall nodule function (Sturz *et al.,* 2000) [63]. Together, these insights position NAB as an emerging and highly valuable microbial group with significant implications for legume productivity, stress resilience, and the broader goals of sustainable agricultural development.

History
In the beginning of the twentieth century, only one nodulating bacterium had been described, *Bacillus radicicola* (subsequently renamed as *Rhizobium*). This development was followed by the discovery of fast- and slow-growing rhizobia, which were subsequently given different generic names (*Rhizobium* and *Bradyrhizobium*). Subsequently, several genera of rhizobia infecting a wide variety of legumes and plant parts were recognised. The rhizobial

genera initially associated with legume nodules were *Allorhizobium, Azorhizobium, Bradyrhizobium, Sinorhizobium (Ensifer), Rhizobium* and *Mesorhizobium* (Zakhia and de Lajudie, 2001) [80]. Currently, the International Committee on Systematics of Prokaryotes (ICSP), Subcommittee on Taxonomy of *Rhizobium* and *Agrobacterium*-Diversity, Phylogenetics and Taxonomy recognises 17 bacterial genera capable of nodulating and fixing atmospheric nitrogen in symbiosis with leguminous. These include 14 α-proteobacterial genera and three genera of β-proteobacteria. The latest genus to be included in this list is *Microvirga,* which is found to encompass three nodulating species in taxonomically separate legume hosts (Ardley *et al.,* 2012) [4].

A landmark discovery in rhizobial ecology was the discovery of the ability of *Burkholderia* and *Cupriavidus,* both belonging to the ß-class of proteobacteria to nodulate legumes (Chen *et al.,* 2001; Moulin *et al.,* 2001) [9, 42]. This gains significance since it was believed that the nodulation trait was exclusively distributed amongst the α proteobacteria to which the classical *Rhizobium* and its related genera belong. A later development in β-proteobacteria was the inclusion of the genus *Herbaspirillum* as a nodulating bacterial species (Valverde *et al.,* 2003) [71]. To encompass this massive development, the terms rhizobia/root-nodulating bacteria (RNB)/legume-nodulating bacteria (LNB) have been coined and have been used by various workers. But the underlying feature of all these terms is the ability of the bacterial species to nodulate and fix atmospheric nitrogen in association with various legume species.

Legume Root Nodules as an Ecological Niche for Bacteria

Legumes (family Fabaceae or Leguminosae) constitute the third largest family of angiosperms plants with a critical importance in human and animal feeding as well as for benefits of agricultural systems. Leguminous and actinorhizal plants are unique among the living beings because they have the characteristic to establish N_2- fixing symbiosis with soil bacteria, collectively known as rhizobia; as a consequence of the plant-bacteria interaction, roots, and occasionally stems, nodules are formed where N_2-fixation occurs (Gresshoff *et al.,* 2023; Lace and Ott, 2018) [26, 36]. A unique feature of rhizobia that sets them apart from plant-associated bacteria is their ability to ultimately become intracellular symbionts within nodule cells.

Nodulation is a complex and specific molecular 'conversation' between the host legume and the rhizobia where the former produce flavonoids in their radical exudates which are detected by rhizobia. Then, flavonoids induce a cascade of nodulation genes in the rhizobia that start the synthesis of nodulation factors, the formation of an infection thread to deliver rhizobia within the epidermal cells of the plant host and, finally the transformation of vegetative rhizobial cells into specialized, N_2-fixing bacteroids (Oldroyd *et al.,* 2011; Downie, 2014) [43, 21].

Nodule Associated Bacteria

Root nodules are typical structures harbouring different types of bacteria (Dekak *et al.,* 2020) [19], which are predominantly colonized with rhizobial species, while other bacteria present inside the nodules are collectively known as non-rhizobial occupants (Preyanga *et al.,* 2021) [48]. They are associated with nodules and plays a functional role in plant growth and development (Figure 1).

A. Rhizobial Occupants of Legume Root Nodules

The rhizobia are a polyphyletic group of bacteria which, in addition to their symbiotic N_2-fixing lifestyle with legumes, are also able to colonize plant roots and even to maintain an endophytic role in non-legume plants (Poole *et al.,* 2018; Schneijderberg *et al.,* 2018) [47, 56]. Classical nodule-forming, N_2- fixing, symbiotic rhizobia belong to the Alphaproteobacteria and Betaproteobacteria classes, albeit members of the Gammaproteobacteria and Actinobacteria have been also reported as symbiotic bacteria (Martínez-Hidalgo and Hirsch, 2017) [40].

Diversity, phylogenetics and taxonomy recognises 17 bacterial genera, namely, *Allorhizobium, Aminobacter, Azorhizobium, Bradyrhizobium, Devosia, Ensifer, Mesorhizobium, Methylobacterium, Microvirga, Ochrobactrum, Phyllobacterium, Rhizobium, Shinella, Sinorhizobium (Ensifer), Burkholderia, Cupriavidus* and *Herbaspirillum,* which are capable of nodulating and fixing atmospheric nitrogen in symbiosis with leguminous. The last three genera that are listed above constitute the beta-proteobacterial group within the rhizobial framework.

The genus *Burkholderi*a comprises of the following nodulating species, namely, *Burkholderia tuberum, B. phymatum* (Vandamme *et al.,* 2002) [72], *B. mimosarum, B. nodosa, B. sabiae, B. caribensis* (Chen *et al.,* 2003) [10], *B. contaminans* (Vanlaere *et al.,* 2009) [73], *B. fungorum* (Coenye *et al.,* 2001) [11], *B. lata* (Vanlaere *et al.,* 2009) [73] and *B. symbiotica* (Sheu *et al.,* 2012) [61]. An interesting feature of rhizobial taxonomy is that often the same genus or even species contains both rhizobial and non-rhizobial strains; for example, the genus *Methylobacterium* contains one rhizobial species M. *nodulans* (Jourand *et al.,* 2004) [32], in addition to several saprophytic species. Similarly, *Cupriavidus* (formerly *Ralstonia taiwanensis*) species is known to have been isolated from nodules as well as clinical samples (Chen *et al.,* 2001) [9]. Therefore, it would be ideal to assess the nodulation potential of a bacterial strain and detect the presence of *nod* and *nif* genes, before assigning it to the broad umbrella of rhizobia.

Table 1: Classification of root nodulating bacteria

Root Nodulating Bacterla	
Classical Rhizobia	Novel Rhizobia
Rhizobium	*Aminobacter*
Allorhizobium	*Burkholderia*
Azorhizobium	*Cupriavidus*
Bradyrhizobium	*Devosia*
Mesorhizobium	*Herbaspirillum*
Sinorhizobium (Ensifer)	*Methylobacterium*
	Microvirga
	Ochrobactrum
	Phyllobacterium
	Shinella

(Selvakumar *et al.,* 2013) [59]

Table 2: Non classical rhizobia associated with various legumes and their features

Bacterial species	Host	Feature
Blastobacter denitrificans	*Aeschynomene indica*	Presence of *nif* HDK gene
Devosia neptuniae	*Neptunia natans*	*nod* D gene of Devosia is closely related to R. *leguminosarum*
Devosia yakushimensis	*Pueraria lobata*	Isolated from the nodules, renodulation not reported
Ensifer adhaerens	*Sesbania grandiflora, Medicago sativa,* etc.	Isolated from multiple genera
Ensifer arboris	*Acacia senegal, Prosopis chilensis*	Isolated from multiple genera
Ensifer fredii	*Glycine* spp., *Vigna unguiculata* and *Cajanus cajan*	Isolated from multiple genera
Ensifer kummerowiae	*Kummerowiae stipulacea*	Single host
Ensifer medicae	*Medicago* spp.	Isolated from multiple hosts
Ensifer meliloti	*Medicago* spp., *Melilotus* spp.	Isolated from multiple genera
Ensifer morelense	*Leucaena leucocephala*	Single host
Ensifer numidicus	*Argyrolobium uniflorum*	Single host
Methylobacterium nodulans	*Crotalaria* spp.	M. *nodulans* contains nod ABC gene and genes encoding structural nitrogenase enzyme
Ochrobactrum lupini	*Lupinus albus*	The nodulating and nitrogen-fixing genes (nod and nif genes) were detected in all the symplasmids using nif H and nod D probes
Ochrobactrum cytisi	*Cytisus scoparius*	Single host
Phyllobacterium trifolii	*Trifolium pratense, Trifolium repens* and *Lupinus albus*	It harbours symbiotic plasmids which have nodulating and nitrogen-fixing genes
Phyllobacterium leguminum	*Astragalus algerianus* and *Argyrolobium uniflorum*	Isolated from multiple genera
Shinella kummerowiae	*Kummerowia stipulacea*	Unable to renodulate the original host plant
Burkholderia caribensis	*Mimosa pudica*, M. *diplotricha*	Single host
Burkholderia dolosa	*Alysicarpus glumaceus*	Only one strain isolated from the host plant
Burkholderia phymatum	*Machaerium lunatum*	Presence of nod ABC shows the capability to produce nod factors to initiate nodulation
Burkholderia tuberum	*Aspalathus carnosa*	Presence of nod ABC shows the capability to produce nod factors to initiate nodulation
Cupriavidus taiwanensis	*Mimosa* spp.	Single host
Cupriavidus necator	*Mimosa caesalpineafolia,* L. *leucocephal Macroptilium atropurpureum,* *P. vulgaris* and *Vigna unguiculata*	Isolated from multiple genera
Herbaspirillum lusitanum	*Phaseolus vulgaris*	Single host

(Selvakumar *et al.,* 2013) [59]
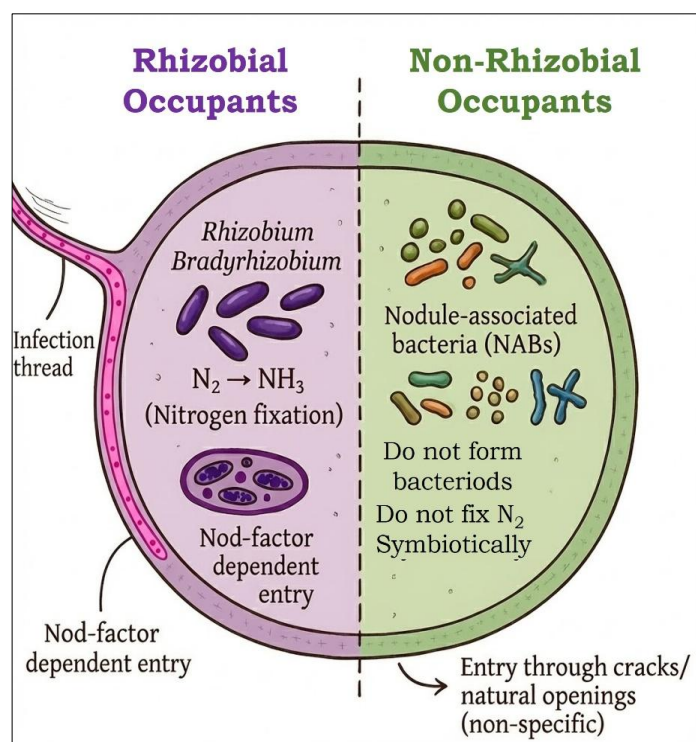
Fig 1: Rhizobial vs Non-Rhizobial Bacterial Entry into Legume Nodules

B. Non-Rhizobial Occupants of Legume Root Nodules

Sturz *et al.,* (1997) [62] made a novel observation that the legume root nodule is known to accommodate several eubacterial genera apart from rhizobia and their population densities are reported to be in the range of 10^4 viable bacteria per gram of fresh nodule tissue. A pioneering observation made by them was that clover root nodules were host to 12 bacteria species other than rhizobia, including eight tissue-specific ones. It was reported that R. *leguminosarum* bv. *trifolii* constituted only 8.8 % of all the root nodule bacteria recovered. The explorations of various legume root nodules exposed a plethora of bacteria that were hither to known to exist in association with legume nodules. The observations that legume root nodules play hosts to diverse microbes like *Bacillus, Streptomyces, Herbaspirillum*, Arbuscular Mycorrhizal Fungi and *Agrobacterium* (Sturz *et al.,* 1997; De Lajudie *et al.,* 1999; Tokala *et al.,* 2002; Valverde *et al.,* 2003; Scheublin *et al.,* 2004) [70, 16, 62, 67] gave rise to a school of thought that they were probably endophytes. But the term 'endophyte' has been much debated, and for a bacterial species to be denoted as a 'true endophyte' more stringent evaluation than mere isolation from surface sterilised plant tissue is suggested (Schulz and Boyle, 2006) [57]. Hence, most non- rhizobial bacteria found in root nodules are commonly referred to as nodule inhabitants. The presence of endophytic bacteria belonging to Alphaproteobacteria, Betaproteobacteria, Actinobacteria and Firmicutes phyla encompassing nine different genera, namely, *Arthrobacter, Bacillus, Bradyrhizobium, Burkholderia, Dyella, Methylobacterium, Microbacterium, Rhizobium and Staphylococcus*, from the nodules of the legume *Lespedeza* sp. grown in two different locations in South Korea was reported by Palaniappan *et al.* (2010) [45]. Most of the isolates they studied showed multiple plant growth promotion activity, i.e. indole acetic acid production, ACC deaminase activity, siderophore production and phosphate solubilisation.

Indigenous legumes in Flanders also show a rich diversity of endophytes, including *Agrobacterium* and *Klebsiella* (De Meyer *et al.,* 2015) [17]. Legumes like cowpea host a variety of endophytes including *Cupriavidus* and *Providencia* (Leite *et al.,* 2017) [37]. *Glycine max* followed by *Vigna radiata, Phaseolus vulgaris* and *Lens culinaris* are the main hosts for nodule associated bacteria (Mohamed Hnini, 2024) [28]. The bacterial species implicated in the nodulation process include *Pantoea agglomerans, Enterobacter kobei, Enterobacter cloacae, Leclercia adecarboxylata, Escherichia vulneris* and *Pseudomonas* sp. Later Zhang *et al.,* (2006) [81] detected the presence of *Pantoea, Erwinia, Salmonella, Enterobacter, Citrobacter* and *Klebsiella* in nodules of the tree species *Conzattia multiflora* grown in Mexico. The presence of *Agrobacterium* strains in nodules, but incapable of nodulating their hosts, has been frequently reported from the nodules of different legumes. Zhang *et al.,* (2006) [81] proved that the *Agrobacterium* strain CCBAU 81181, which was originally isolated from the root nodules of *Onobrychis viciifolia*, and a symbiotic strain of *Sinorhizobium meliloti* CCBAU 10062 could actually co-inhabit the root nodules of *Melilotus dentatus*. Kan *et al.,* concluded from a study of 61 root nodule isolates from diverse legumes, namely, *Vicia, Oxytropis, Medicago, Melilotus* and *Onobrychis* species grown in Qinghai–Tibet plateau, that in addition to nodulating genera like *Rhizobium leguminosarum*, S. *meliloti, Sinorhizobium fredii, Mesorhizobium* sp. and *Phyllobacterium* sp., two non-symbiotic groups related to Agrobacterium and Enterobacteriaceae were present in their nodules. Selvakumar *et al.* (2008) [58] reported the presence of diverse plant growth promoting strains of bacteria such as *Bacillus thuringiensis, Enterobacter asburiae* and *Serratia marcescens* from the nodules of the legume Kudzu (*Pueraria thunbergiana*) grown in the Indian Himalayan Region. Dashti *et al.,* (2009) [14] made an unusual finding that the surfaces of root nodules of *Vicia faba* and *Lupinus albus* were colonised by bacterial consortia that utilised oil and fixed nitrogen. This finding has immense value in the realm of nitrogen-poor desert soils where anthropogenic oil spills are quite common.

Table 3: Some non-rhizobial bacteria associated with legume root nodules and their features

| Bacterial species	Host plant	Features
Agrobacterium -like strains	*Phaseolus vulgaris, Acacia, Prosophis, Chamaecrista*	Nitrogen-fixing genes were detected
Labrys neptuniae	*Neptunia oleracea*	Novel species
Microbacterium sp. and *Starkeya* sp.	Spontaneous legumes	Presence of nif H-like gene detected
Bacillus megaterium, Brevibacillus choshinensis and *Microbacterium trichothecenolyticum*	*Medicago sativa*	Plant growth promotion traits
Bacterial isolates with maximum similarity to *Bacillus subtilis, Bacillus simplex* and *Agrobacterium tumefaciens*	*Vigna radiata*	IAA production, P solubilisation

(Selvakumar *et al.,* 2013) [59]

Mode of Entry of Nodule Associated Bacteria

Bacterial genera most frequently isolated from inside nodules are also the most commonly found as root endophytes (including both legumes and non-legumes), suggesting that colonization of nodules does not rely on microbial specific traits others than the ones required for root colonization. Sites for primary colonization and entry into the plant of non-symbiotic bacterial endophytes are undifferentiated tissues above the root tips and the points of emergence of lateral roots, as also described for rhizobia (Reinhold-Hurek and Hurek, 1998) [50]. This first step in the tissue entry process of non-symbiotic endophytes also involves root adsorption and bacterial proliferation, forming biofilm structures at the surface of roots (Compant *et al.,* 2010; Reinholdt-Hurek and Hurek, 2011) [12, 51]. In fact, they use epidermal junction between root hair and adjacent epidermal cells, or disrupted endodermal cell layers resulting from the emergence of developing lateral roots, the mechanism known as "crack entry" (Figure 2). Root hairs also represent a site for endophytic bacteria entry. For *Pseudomonas* spp. the main entrance for endophytic colonization of olive are root hairs. In *Vigna radiata*, the invasion of infection threads by *Pseudomonas*

and *Klebsiella* strains led to nodule colonization when co-inoculated with host-nodulating *Ensifer adhaerens*.
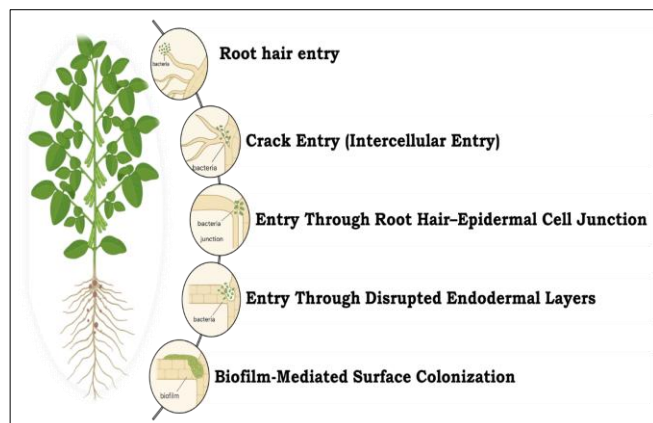
Fig 2: Mode of entry of nodule associated bacteria in legume plants

Functional Role of Legume Root Nodule Associated Bacteria

In contrast to the rhizobia whose role is largely confined to diazotrophy, non rhizobial nodule occupants seem to have a diverse influence on the plant survival, nodulation and growth promotion and yield (Selvakumar *et al.,* 2008) [58]. In general, the non-rhizobial bacteria are thought to synergistically act with rhizobia and increase nodulation and yield possibly by production of growth hormones like IAA production, solubilization of nutrients, N fixation and siderophore production (Figure 3). The symbiotic effectiveness of rhizobia can be improved by co-inoculation with suitable non-rhizobial beneficial bacteria in most legume. Nodule associated bacteria promote plant growth through mechanisms such as phytostabilization, biofertilization, biocontrol and recuperation of degraded soils (Mitra *et al.,* 2015) [41]. Specifically, endophytic bacteria residing in legume nodules demonstrate potential as biofertilizers. In view of this, researchers tend to focus their attention towards the isolation and characterisation of non-rhizobial bacteria from legume nodule and utilising these strains to improve nodulation, crop growth and yield.

1. Biofertilization

1.1 Nitrogen fixation by Nodule associated bacteria

Nitrogen (N) is an essential macro-element that is crucial for plant growth and development. Legumes are able to meet most of this requirement through their symbiotic association with rhizobia where atmospheric N is converted to ammonium in root nodules and transported within the plants as glutamine. Nitrogen fixation is a critical process for sustainable agriculture, and various bacterial strains have been identified for their ability to fix atmospheric nitrogen either as free-living organisms or as endophytes within legume nodules.

Studies have documented the nitrogen-fixing capabilities of *Serratia* sp., *Herbaspirillum* sp., *Paenibacillus* sp., *Arthrobacter* sp., *Klebsiella* sp., *Pseudomonas* sp., *Bacillus* sp., *Bosea* sp., and *Enterobacter* sp. strains isolated from legume nodules (Valverde, 2003; Selvakumar *et al.,* 2008; Ibáñez *et al.,* 2009; Knežević *et al.,* 2021; Debnath *et al.,* 2023) [70, 58, 18]. Recent investigations suggest that nodule associated bacteria contribute substantially to nitrogen fixation, potentially accounting for up to 47% of the total

nitrogen fixed by bacteria. This contribution spans both symbiotic and free-living states, underscoring the multifaceted role of NREs in nitrogen cycling within the biosphere.

1.2 Nodulation enhancement

The synergistic effects of N-fixing rhizobia with other beneficial microbes on plants has been reported, little information is available on the mechanisms that improve nodulation and legume growth. The infection of legume roots by rhizobia and formation of N fixing nodules involves a complex signalling between plant and rhizobium (Sharma *et al.,* 2020) [60]. Nodule associated bacteria do not independently induce nodules, they enhance nodule formation when co-inoculated with suitable rhizobia, displaying various plant growth-promoting properties (Li *et al.,* 2012) [38]. It is interesting to highlight the case of some *Agrobacterium* strains, including those isolated from common bean root nodules and other legumes, that were reported as having the capacity to nodulate legume plants (De Lajudie *et al.,* 1999; Valdes *et al.,* 2014; El Attar *et al.,* 2019) [16, 69, 23]. This nodulation ability may be attributed to the acquisition of symbiotic genes via lateral gene transfer, as suggested in various studies. Supporting this idea, some Agrobacterium strains were found to carry symbiosis-specific genes (e.g., *nif* H and *nod* A), similar to those present in well-known rhizobia legume symbionts (Cummings, 2009; Rincón Rosales *et al.,* 2009; Youseif *et al.,* 2014) [13, 52, 72].

The co-inoculation of rhizobia (*Mesorhizobium ciceri* strain CC1192) and strains of Nodule associated actinobacteria (CP56 *Microspora* sp., CP84B *Actinomadura* sp., CP200B *Streptomyces* spp., CP21A2 *Streptomyces* spp.) was found to consistently increase the nodulation, growth and grain yield of chickpea in a largely nitrogen-free sand -vermiculite system. Multiple mechanisms may have contributed to the responses due to the treatment with actinobacteria. These include changes in the root architecture both in the absence and presence of rhizobia, faster and increased growth of the rhizobia in response to actinobacterial compounds and increased secretion of specific root exudates including flavonoids, increased attraction of rhizobia to the roots, increased biofilm production, and the higher levels of expression of *nod* genes leading to earlier nodulation and increased nodule mass. Together with a higher level of expression of *nif* and *fix* genes for some actinobacteria strains, their application resulted in higher grain yields. If duplicated in the field, the application of actinobacteria should increase nitrogen inputs into the farming system (Xu *et al.,* 2022) [77].

1.3 Phosphate solubilization for enhanced phosphorus availability

Phosphorus is a crucial element for plant growth that is often at low levels in the soils or present but not available for plants. Phosphate-solubilizing bacteria (PSB) play an essential role by transforming the insoluble molecules. It was reported that nodule associated bacteria can solubilize insoluble phosphates during initial colonization of roots, thereby improving phosphorus availability in the soil (Kai-yuan *et al.,* 2023) [33]. Solubilization of inorganic P phenomenon is often associated with a lowering in pH by microorganisms. Phosphate solubilization has been considered one important trait that bacteria display to

promote plant growth, since phosphate is often found as insoluble salts which are not available to the plant. Usually, phosphate solubilization is associated with soil acidification which dissolves the inorganic insoluble phosphate salts.

1.4 Production of siderophores
Certain strains of *Bacillus* within nodule endophytic bacteria contribute to plant growth by producing siderophores, which are low-molecular-weight metabolites that chelate iron, making it available to plants. This production has significant implications for competitively inhibiting the growth of phytopathogens (Maheshwari *et al.,* 2019; Dhole *et al.,* 2022) [39, 20]. In addition to these traditional biofertilization mechanisms, recent studies have shed light on the role of nodule associated bacteria (NAB) in enhancing plant health and productivity (Tokgoz *et al.,* 2020) [68].

1.5 Production of plant growth regulators
Plant hormones, also known as phytohormones, are natural organic compounds produced by plants, and they play the role of chemical messengers that influence plant growth and its interaction with the environment. These plant growth regulators (PGR) could be produced in some specific parts of the plant, and then they are transported to different organs, where they can influence many physiological, biochemical, and morphological processes. Nodule associated bacteria has the capability to synthesize various hormones like auxins Indoleacetic Acid (IAA), gibberellins, cytokinins etc (Orozco-Mosqueda *et al.,* 2023) [44].

2. Biocontrol
Microorganisms, particularly those with plant growth-promoting characteristics, offer a promising approach for mitigating pathogen pressure. Upon colonizing plant tissues, they participate in the competitive exclusion of pathogens by strategically occupying preferred sites of invasion, leading to the effective utilization of nutrients that curtails pathogen invasion. Nodule endophytic bacteria strains exhibiting significant lytic enzyme activities may play a crucial role in protecting plants against various pathogenic fungi, as indicated by studies on *Botrytis cinerea, Fusarium oxysporum, Pythium ultimum, Phytophthora sp., and Rhizoctonia solani* (Yasmin *et al.,* 2016; Chen *et al.,* 2020) [78, 8]. In a study by Bahroun *et al.,* 2018 [5], three strains, namely *Rahnella aquatilis* B16C, *Pseudomonas yamanorum* B12, and *Pseudomonas fluorescens* B8P, identified as nodule associated bacteria, showed *in vivo* biocontrol potential against *Fusarium solani* root rot in three Faba bean cultivars under greenhouse conditions. Recent reports that soybean nodules harbour a large number of nodule associated bacteria from diverse genera in addition to traditional resident rhizobia. The nodule associated bacterial isolates exhibited their potential antagonistic activities against two economically important fungal plant pathogens *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* (Tokgoz, 2022) [68].

Nodule associated bacteria also produced antibiotics, with certain strains, such as *Bacillus*, synthesizing compounds like circulin, colistin, and polymyxin. These compounds exhibit inhibitory effects on both Gram-positive and Gram-negative bacteria, as well as numerous pathogenic fungi. Additionally, several bacteria, including endophytes, can produce bacteriocins-specific proteins inhibiting the activity of strains within the same bacterial species or related species

(Vasileva *et al.,* 2019) [74]. Chitin is a major component of fungal cell walls and biocontrol of phytopathogens could rely on chitinase activity. Sanchez-Cruz, 2019 reported that nodule associated *Serratia* sp. strains (NOD 3 and NOD7) showed chitinase activity.

3. Metal phytostabilization
Industrial and mining activity releases toxic metal/loids into the environment, such as As, Cd, Cu, and Pb, all of them very harmful to human health and most living beings (FAO and ITPS, 2015). These metals have also been repressing the enzymatic activity of the soils, causing a decrease in the growth and respiration of the populations of microorganisms, thus altering the diversity present in the rhizosphere. It will also alter the plant adaption and growth (Abdu *et al.,* 2017; Aponte *et al.,* 2020) [7, 3].

Microorganisms have an important role in pollutant detoxification and heavy metal plant stress resistance (Caracciolo and Terenzi, 2021) [6]. Soil microorganisms have developed different resistance mechanisms, such as metal biosorption, bioaccumulation, modification of metal chemical state (methylation) or production of chelating compounds, particularly siderophores and biosurfactans, that cause a lowering in metal availability for plants (Verma and Kuila, 2019) [75], then diminishing plant metal content (Caracciolo and Terenzi, 2021) [6]. The use of legumes and associated rhizobia is an interesting tool to fight against soil metal contamination, since legume interacting with rhizobia have the ability to accumulate high concentrations of metals particularly in roots, with low levels of translocation to shoots, without disturbing plant growth (Jach *et al.,* 2022) [30].

Regarding the accumulation of metals/loids in the roots, inoculation with *Pseudomonas* or *Pseudomonas-Ensifer* (isolated from the root nodules) couples promoted metal accumulation and the highest levels of As, Cd, Cu and Zn were measured in *Medicago sativa* roots inoculated with the consortium of the four strains (CSN). In that way, inoculation with *Pseudomonas* strains enhanced the metal phytostabilization potential of *M. sativa* plants in soils with moderate to high levels of metals, without negative effects on plant growth.

4. Recuperation of degraded soils
The increase of the global human population demands greater extensions of arable land to cover the production of food, fodder, fibre and biomass which, in turn, is causing biotic and abiotic stresses to the soil (Dubey *et al.,* 2020). About 30% of the global soil area is degraded or contaminated due to anthropogenic activities. Soil degradation implies reduction and even loss of its physicochemical and biological characteristics, poor agricultural practices and the use of xenobiotic contaminants being the most common cause of the loss of soil quality (Schloter *et al.,* 2018) [55]. Among the cultural practices that generate soil degradation are the burning of the vegetation cover, deforestation and monoculture farming, leading to a loss of organic matter by reducing the soil microbial activity and biomass (Kaschuk *et al.,* 2010) [34].

NAB is being used as a biotechnological tool for the recovery of degraded soils and of those contaminated with xenobiotic compounds. In association with NAB, legumes manage to tolerate high concentrations of heavy metals, improving the phytoremediation process, preventing the entry of heavy metal into the food chain (Jian *et al.,* 2019)

[31] and restoring soil fertility by increasing the nitrogen content of the soil (Gomez-Sagasti *et al.,* 2010) [25].

Phytoremediation using NAB is a low-cost technology that can be applied to the restoration of contaminated soils (Ahemad and Khan, 2011; Tetard-Jones and Edwards, 2016) [1, 66]. The recovery of degraded soils can be facilitated by using microorganisms that increase the vegetation cover. Several studies have shown that NAB has a critical role in the restoration of marginal, degraded and contaminated soils in the prevention of soil erosion and in the afterwards reforestation events. Germaine *et al.* (2006) [24] showed that the inoculation of *Pisum sativum* with *Pseudomonas putida* VM1450 diminished the content of the herbicide 2, 4-D in the soil and prevented its accumulation in the aerial part of the plant. Similarly, Wani and Khan (2010) [76] showed that treatment with *Bacillus* sp. PSB10 of *Cicer arietinum* seeds mitigated the toxic effects of the hexavalent chromium present in contaminated soils. Soils contaminated with hydrocarbons were successfully recovered using *Lotus corniculatus* and *Oenothera biennis* plants inoculated with *Rhizobium*, *Pseudomonas*, *Stenotrophomonas* and *Rhodococcus* NAB (Pawlik *et al.,* 2017) [46]. Kong *et al.* (2017) [35] showed that the co-inoculation of *Sinorhizobium meliloti* and *Pseudomonas brassicacearum* Zy-2-1 improved the growth of *Medicago lupulina* in the presence of toxic Cu^{2+} concentrations. The ability of NAB isolated from root nodules of legumes in the recovery of degraded soils allows us to infer they have excellent potential to improve soil health and plant health.
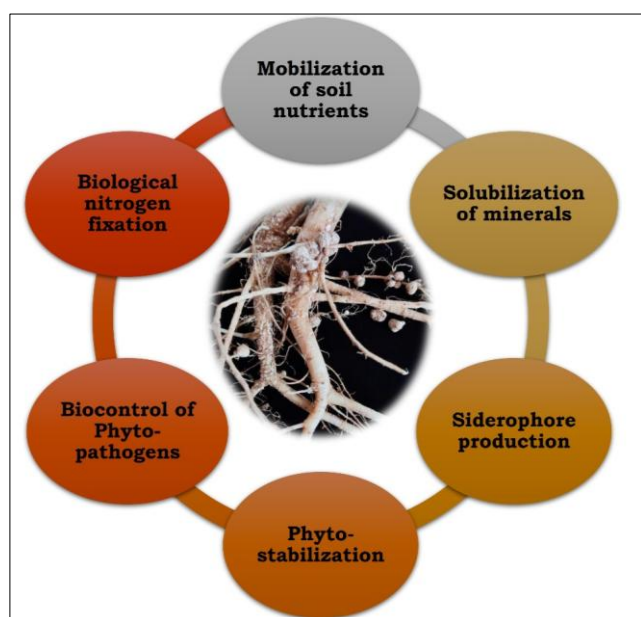
Fig 3: Functional role of legume root nodule associated bacteria

Conclusion
Nodule harbours not only symbiotic 'rhizobia' but also a wide plethora of non- rhizobial organisms that play both well-established and cryptic roles in plant metabolism. Beyond rhizobia, non-rhizobial endophytes contribute to the ecological resilience of legumes. Nodule associated bacteria co-inoculated with Rhizobial strains has a very significant role in biofertilization, biocontrol, metal phytostabilization and recuperation of degraded soils. Like rhizospheric bacteria, Nodule associated bacteria have direct and indirect mechanisms of plant growth promotion, and their utilization, whether alone or in co-inoculation with rhizobia,

could constitute an ecological and healthy alternative to synthetic fertilizers for legume and non-legume plants. However, the environment in the rhizosphere is quite different to that in the nodule interior, so that differences in the biotic and abiotic stresses inside and outside the nodule (soil type, temperature, pH, oxygen concentration, nutrient availability, etc.) could affect the survival and lifestyle of nodule associated bacteria.

Future Prospects
Determining the cross-inoculation potential of novel rhizobial strains with various cultivated legumes to understand their compatibility and symbiotic efficiency is an important aspect which needs to be addressed. This includes assessing the specificity of nodule-associated bacteria, defining their cross-inoculation groups, and supporting these insights with metagenomic analyses. Further, the diazotrophic benefits of the newly identified rhizobial–legume associations has to be quantified using both isotopic and non-isotopic approaches. The nitrogen-fixing potential of the novel strains can be evaluated through classical methods such as the Acetylene Reduction Assay (ARA) and ^{15}N dilution studies. Further research to explore the role of non-rhizobial bacteria as co-inoculants, particularly in consortium-based formulations with rhizobia, to enhance nodulation efficiency and overall symbiotic performance is required.

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