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Exploiting Rhizobia for the Biological Management of Soilborne Fungal Diseases

K. Manasa[1], Challa Yashaswini[2], K. Gopika[2] and, Adluri Prashanth[3]

Abstract: -

Rhizobia are a group of microorganisms renowned for their ability to colonize plant root surfaces and form symbiotic relationships with leguminous plants. Besides their essential role in biological nitrogen fixation, they also contribute to enhanced plant growth and reduced incidence of crop diseases. Rhizobia have been shown to inhibit a variety of soilborne fungal pathogens from genera such as *Fusarium*, *Rhizoctonia*, *Sclerotium*, and *Macrophomina*. Their antagonistic effects are primarily linked to the production of antibiotics, hydrocyanic acid (HCN), mycolytic enzymes, and siderophores, especially under iron-deficient conditions. Additionally, rhizobia can trigger systemic resistance in plants and boost the expression of defense-related genes, effectively enhancing plant immunity against pathogens. Treating seeds with suitable rhizobial strains can lead to the production of phenolic compounds, isoflavonoid phytoalexins, and activation of key defense enzymes like L-phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), peroxidase (POX), and polyphenol oxidase (PPO), which are part of the phenylpropanoid and isoflavonoid biosynthetic pathways. Developing rhizobial inoculants that combine nitrogen-fixing ability with pathogen suppression offers potential for improving plant health and boosting agricultural productivity. This review consolidates current knowledge on the biocontrol capabilities of rhizobia and aims to highlight research gaps and effective approaches for future studies in this field.

Introduction

Legume crops are essential for both nutritional security and sustainable agriculture across the globe. They play a crucial role in improving soil fertility by fixing atmospheric nitrogen. Between 2006 and 2008, legumes were cultivated on approximately 61.5 million

***K. Manasa*[1]*, Challa Yashaswini*[2]*, K. Gopika*[2] *and, Adluri Prashanth*[3]**

[1]*Assistant Professor, Department of Agricultural Microbiology, PJTSAU, Agricultural College, Warangal*

[2]*Assistant Professor, Department of Plant Pathology, PJTAU, Agricultural College, Warangal*

[3]*Scientist, Department of Plant Pathology, Research Station: JVRHRS Malyal, SKLTGHU*

hectares worldwide, producing about 46.5 million tons. Among food legumes, dry beans (*Phaseolus* spp.) accounted for 46% of the cultivated area, followed by chickpeas and cowpeas at 18% each. In terms of production, dry beans also led with 46%, while chickpeas contributed 22%. Despite their importance, legumes occupy only about 10% of the area used for cereal crops globally and are mainly grown in rainfed, low-input systems. As a result, their average yield in 2008 was just 0.86 t/ha-about one-fourth that of cereals, which averaged 3.54 t/ha. Nonetheless, in recent years, production of food legumes has seen substantial growth in both developing and developed nations. Nitrogen is a vital nutrient required in large amounts for plant growth, but its availability in soil often limits crop productivity. While atmospheric nitrogen (N_2) makes up 78% of air, plants cannot directly absorb it. Diazotrophic bacteria, particularly rhizobia, convert atmospheric nitrogen into plant-usable ammonium through biological nitrogen fixation (BNF). Rhizobia form symbiotic relationships with legumes and significantly enhance soil nitrogen levels. These symbioses can fix between 13–360 kg of nitrogen per hectare annually, though fixation efficiency varies depending on legume species, rhizobial strain, and soil conditions. For instance, nitrogen fixation can range from 17–450 kg/ha in soybean, 37–206 kg/ha in groundnut, 119–140 kg/ha in black gram, and 68–88 kg/ha in pigeonpea under ideal conditions. Forage and tree legumes also significantly contribute to global nitrogen fixation. However, legume crops are vulnerable to a variety of fungal diseases caused by pathogens such as *Fusarium oxysporum* f. sp. *ciceri*, *Ascochyta rabiei*, *Botrytis cinerea*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. Fusarium wilt alone causes 10–15% yield losses annually and can lead to losses up to 60–70% during severe outbreaks. Disease management mainly relies on resistant varieties and fungicide applications, but excessive use of fungicides leads to environmental pollution and disrupts beneficial soil microbes. To address this, biocontrol agents (BCAs) have been developed and tested for managing these pathogens. While many show potential against fungal diseases, only a limited number have been commercialized. A notable success is the use of fluorescent pseudomonads to control take-all disease in wheat, resulting in a 27% yield increase in field trials.

Rhizobia and biocontrol

The use of Rhizobium inoculation continues to play a key role in enhancing global production of soybeans and other legumes. While its primary function is to facilitate symbiotic nitrogen fixation in legumes, many rhizobial species have also been found to promote plant growth and suppress a variety of soilborne pathogens-including *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium* species-in both leguminous and non-leguminous crops. For instance, applying Sinorhizobium meliloti, *Rhizobium leguminosarum* bv. *viceae*, and *Bradyrhizobium japonicum* either as seed coatings or soil drenches has been shown to lower disease incidence from *M. phaseolina*, *R. solani*, and *Fusarium* spp. in okra. Similarly, seed inoculation with *R. leguminosarum* bv. *phaseoli* significantly reduced root rot in beans artificially infected with *Fusarium solani* f. sp. *Phaseoli*. Several studies have reported antagonistic effects of rhizobial and bradyrhizobial isolates on *M. phaseolina*, the causative agent of charcoal rot in groundnut. In one case, treating seeds with strain R12 of *R. leguminosarum* bv. *viceae*, isolated from lentils, not only boosted seedling emergence but also reduced Pythium damping-off in field pea and sugar beet. Likewise, strains R20 and R21, isolated from pea, showed comparable effectiveness against Pythium in peas. Rhizobium species have also been found effective in managing chickpea diseases. Out of 42 nitrogen-fixing and plant growth-promoting *Rhizobium* strains, 24 were effective in inhibiting *R. solani* in vitro. In pot trials, these isolates helped reduce root rot in chickpeas. Additionally, *Rhizobium japonicum* (*B. japonicum*) strains demonstrated the ability to suppress *F. solani* and *M. phaseolina* in both culture and soil. Inoculating soybean seeds with these strains improved germination and decreased root rot severity in both pot and field experiments. Some *B. japonicum* strains that produce rhizobiotoxin have also been found to protect soybeans from *M. phaseolina* infections.

Interactive effect of Rhizobium with other PGPR in biocontrol

Research indicates that combining multiple plant growth-promoting rhizobacteria (PGPR) strains-particularly those with varying mechanisms for promoting plant growth or suppressing soilborne pathogens-tends to be more effective than using a single-strain inoculant. This synergistic interaction is especially evident when PGPR are used alongside arbuscular mycorrhizal (AM) fungi**.** For example, filtrates from three wild rhizobial strains used in conjunction with AM fungi demonstrated strong biocontrol effects against damping-off and root rot in faba beans caused by *Rhizoctonia solani*, *Fusarium solani*, and other *Fusarium* species. Similarly, co-inoculation of AM fungi and *Rhizobium leguminosarum* has been shown to protect beans from *Botrytis fabae*. Another study by Akhtar and Siddiqui (2008) found that applying *Rhizobium* together with *Glomus intraradices* and *Pseudomonas striata* significantly reduced root rot in chickpeas caused by *Meloidogyne incognita* and *M. phaseolina*, while also improving chlorophyll content, pod count, and the uptake of nitrogen, phosphorus, and potassium—resulting in enhanced overall plant growth. Contrasting results were reported in a study where individual applications of *R. leguminosarum* and AM fungi (*Glomus hoi* and *Glomus fasciculatum*) were more effective than combined inoculation in controlling Fusarium wilt in chickpeas. In another study, dual inoculation with *Rhizobium* and *Trichoderma* was shown to suppress damping-off and root rot diseases, which led to increases in yield-related traits like branches per plant, number of pods, seeds per pod, seed weight, and total seed yield in crops such as broad beans, chickpeas, and lupins. Application of *Rhizobium* in combination with *Trichoderma harzianum* also helped reduce the incidence of collar rot in groundnut caused by *Sclerotium rolfsii* while improving plant metrics such as root and shoot length and biomass. Emerging studies on cyanobacteria as beneficial partners in legume production have created new research opportunities. Cyanobacterial species like *Anabaena laxa* and *Calothrix sp.* were found to enhance yields, nitrogen fixation, and leghaemoglobin levels when co-inoculated with rhizobia. Furthermore, *Kumar et al. (2010)* reported that dual inoculation with *Sinorhizobium fredii* KCC5 and *Pseudomonas fluorescens* LPK2 significantly suppressed Fusarium wilt in pigeon pea. This treatment increased nodule and pod numbers, root and shoot length, and overall biomass. They observed aggressive colonization of roots, disintegration of fungal hyphae, and breakdown of fungal conidia, which led to effective control of *Fusarium udum*. Likewise, using *Bradyrhizobium japonicum* along with *P. fluorescens* and *Pseudomonas aeruginosa* offered protection to tomato plants against root rot and root-knot diseases.

Mechanisms involved in biocontrol

Rhizobia suppress pathogenic fungi through various mechanisms such as mycoparasitism, production of antibiotics and antifungal compounds like hydrogen cyanide, and the release of siderophores that lead to competition for iron. They also compete with pathogens for nutrients, stimulate plant defense systems, and enhance plant growth, thereby reducing the plant's vulnerability to disease.

Mycoparasitism

Several studies have documented that various rhizobial strains can parasitize fungal hyphal tips or suppress reproductive structures such as sclerotia and zoospores. Rhizobia present in the plant rhizosphere may inhibit the growth of harmful fungi by covering their hyphal tips and subsequently causing hyphal lysis through antibiotic production. For instance, *Bradyrhizobium japonicum* has been shown to inhibit the germination and development of *Sclerotium rolfsii* sclerotia. Fast-growing rhizobial strains are generally more effective at suppressing white sclerotia of *S. rolfsii*, while slow-growing strains of *Bradyrhizobium* have also demonstrated the ability to inhibit mycelial growth and sclerotial development of *Rhizoctonia solani*. Additionally, rhizobia have been shown to reduce fungal mycelial dry weight and suppress the survival of *Phytophthora cinnamoni* zoospores, thereby protecting host plants even in non-sterile, pathogen-infested soils.

Antibiotic production

The production of antifungal antibiotics is recognized as a key mechanism by which rhizobia suppress fungal pathogens. Numerous studies have documented the ability of various rhizobial strains to produce such antibiotics. For example, *Rhizobium leguminosarum* strains have been found to produce bacteriocins-classified as small, medium, or large based on their size. One study revealed that the symbiotic plasmid pRL1J1 in *R. leguminosarum* carries genes not only for nodulation and nitrogen fixation but also for bacteriocin synthesis. Additionally, *R. leguminosarum* bv. *trifolii* has been shown to produce the peptide antibiotic trifolitoxin (TFX). *Bradyrhizobium* species produce rhizobitoxine, which has demonstrated the ability to reduce *Macrophomina phaseolina* infections in crops such as soybean and groundnut. Other strains of *R. leguminosarum* (bv. *viciae*, *trifolii*), *R. meliloti*, *R. trifolii*, *Sinorhizobium meliloti*, and *Bradyrhizobium japonicum* have also been reported to secrete antibiotics effective against a range of plant pathogens.

Induction of plant defense mechanisms

Induced systemic resistance

Induced systemic resistance (ISR) is recognized as an important mechanism by which various microorganisms, including rhizobia, help suppress fungal diseases. Rhizobia can activate a plant's defense responses when the plant is exposed to fungal pathogens. This activation involves the production of defense-related enzymes, phenolic compounds, flavonoids, and phytoalexins. Phenolics, in particular, contribute significantly to limiting pathogen development and spread by acting as structural barriers, signaling molecules, and modulators of pathogenicity. For example, pre-inoculating broad bean plants with *Rhizobium leguminosarum* bv. *viceae* led to increased levels of total and free phenolics after infection with *Botrytis fabae*. Similarly, when *Rhizobium* was applied to non-leguminous plants such as rice, it induced the accumulation of phenolic acids like gallic, ferulic, tannic, and cinnamic acids, which correlated with reduced sheath blight caused by *Rhizoctonia solani*. Rhizobia also trigger the synthesis of phytoalexins-antimicrobial compounds produced in response to pathogen attack. In chickpea, the isoflavonoids medicarpin and maackiain act as primary defense compounds. Their production has also been noted in alfalfa and common bean following rhizobial inoculation. In peas, the phytoalexin 4-hydroxy-2,3,9-trimethoxy pterocarpan, induced by *R. leguminosarum* bv. *viciae*, has shown protection against *Fusarium solani* f. sp. *pisi*. In soybeans, cyclic β-glucans produced by *Bradyrhizobium* have been linked to the induction of glyceollin, a potent phytoalexin. In addition, rhizobia enhance the expression of genes associated with plant defense, leading to the production of enzymes such as L-phenylalanine ammonia lyase, chalcone synthase, isoflavone reductase, peroxidase, and polyphenol oxidase. These enzymes help mitigate pathogen impact. For instance, pretreatment of chickpea with the rhizobial strain PchDMS before infection with *F. oxysporum* boosted levels of several defense markers, including peroxidases, polyphenol oxidases, and isoflavonoids like formononetin and biochanin A. Further evidence of ISR includes reports of resistance induction in common bean against bacterial blight following inoculation with *R. leguminosarum* bv. *phaseoli*, and increased defense enzyme activity in peas exposed to *Orobanche crenata* after rhizobial inoculation. Additionally, purified lipopolysaccharides from *Rhizobium etli* have been shown to trigger systemic resistance against nematodes, with mutants lacking lipopolysaccharide biosynthesis genes failing to elicit the same response.

Rhizobial formulations

After identifying the most effective rhizobial strain with both strong nitrogen-fixing capacity and antifungal properties, the next critical step is to develop a suitable inoculum formulation. This formulation plays a key role in determining the overall effectiveness and success of the inoculant in agricultural use. The primary goal is to ensure that the selected microbial strain remains viable in high numbers and retains its beneficial traits during storage, up until field application. Commercially, Rhizobium inoculants are typically available in two forms: solid (carrier-based) and liquid. Solid inoculants are produced by blending a concentrated rhizobial culture with an appropriate carrier material. The selection of carrier depends on factors such as cost, availability, moisture retention, pH buffering ability, and how well it supports the survival of rhizobial cells. A wide range of materials is used globally as carriers, including soil-based options like peat, charcoal, volcanic pumice, and clay; organic materials such as compost and plant residues; and inert substances like perlite, vermiculite, kaolin, bentonite, and silicates. Liquid formulations generally consist of rhizobial cultures suspended in broth, mineral or organic oils, or oil-in-water emulsions. As bio-inoculants become more widely adopted, innovation in formulation technology has focused on improving cost-efficiency, product stability, and shelf life. For example, Rebah et al. (2007) highlighted the use of wastewater sludge-within permissible heavy metal limits-as both a growth medium and dehydrated carrier material, offering a low-cost alternative for inoculant production. Moreover, the incorporation of polymer-based carriers, such as carboxymethyl cellulose with starch, alginate, polyvinylpyrrolidone, and polyethylene glycol, has been shown to enhance the stability and longevity of microbial products. Recently, biofilm-based inoculant formulations have gained attention due to their ability to provide a protective environment for microbial communities, helping them withstand various biotic and abiotic stresses. These biofilms can form on inert carrier materials or within a fungal matrix that encapsulates bacterial cells. Studies have demonstrated improved plant growth outcomes when using biofilm-formulated inoculants compared to traditional ones. For example, biofilms developed with *Trichoderma* or cyanobacteria such as *Anabaena*, in combination with rhizobia and other beneficial microbes, have shown promising results as plant growth promoters and biocontrol agents in crops like rice, cotton, soybean, chickpea, and lentil.

Future prospects and challenges

Beneficial microorganisms with multiple advantages are increasingly sought after as inoculants in global agriculture. Rhizobia, known for their nitrogen-fixing and plant growth-promoting properties, are ideal candidates for genetic modification to express antibiotic-producing genes from other microbes. For example, Krishnan et al. (2007) demonstrated that *R. etli* USDA9032, engineered to produce phenazine, displayed antifungal activity, though it suffered a notable reduction in its nitrogen-fixing capacity. Further research is essential to develop rhizobial strains that can both effectively nodulate plants and suppress diseases. Another promising approach is rhizosphere engineering, where plants are genetically modified to release compounds that promote the growth of beneficial microorganisms. The combination of transgenic plants and modified rhizobia could enhance root colonization, pathogen protection, and overall plant growth and yield.

An important area for improvement is the rhizosphere competence and survival of inoculated rhizobia, along with a deeper understanding of their ecological physiology. This knowledge will be crucial for creating effective technologies that farmers can use. Additionally, the availability of high-quality carrier materials and advanced formulations is key to the success of inoculants in the field. New formulation technologies include:

- **Polymer-based formulations**, which have shown good storage stability.
- **Water-in-oil emulsions**, which are beneficial for microorganisms sensitive to desiccation.
- **Biofilm-based formulations**, where fungal or bacterial partners help protect microorganisms, ensuring their survival and growth under challenging soil conditions, including biotic and abiotic stress.
- The application of nanotechnology, which could revolutionize biofertilizers by encasing bacterial cells in nanostructures to improve stability and surface area. Nanoformulations may enhance the resilience of biofertilizers against desiccation, temperature, and UV degradation.

However, further research is needed to address biosafety concerns related to transgenic organisms and nanoformulations. There is a clear need for focused efforts to better understand symbiotic relationships for more effective applications in marginal or polluted lands. The potential role of other beneficial plant growth-promoting (PGP) microbes as co-inoculants or elicitors remains an under-explored but promising area, with limited information available thus far.

References

1. Akhtar MS, Siddiqui ZA (2008) Biocontrol of a root-rot disease complex of chickpea by Glomus intraradices, Rhizobium sp. And Pseudomonas straita. Crop Prot 27:410-417
2. Krishnan HB, Kang BR, Hari Krishnan A, Kim KY, Kim YC (2007) Rhizobium etli USDA9032 engineered to produce a phenazine antibiotic inhibits the growth of fungal pathogens but is impaired in symbiotic performance. Appl Environ Microbiol 73:327-330
3. Kumar H, Bajpai VK, Dubey RC,Maheshwari DK, Kang SC (2010)Wilt disease management and enhancement of growth and yield of Cajanus cajan (L) var. Manak by bacterial combinations amended with chemical fertilizer. Crop Prot 29:591-598

4. Rebah FB, Prévost D, Yezza A, Tyagi R (2007) Agro-industrial waste materials and wastewater sludge for rhizobial inoculant production: a review. Bioresour Technol 98:3535–3546.