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Biological Control of Diseases Caused by *Rhizoctonia solani* AG-4 in Bean (*Phaseolus vulgaris* L.) using Rhizobacteria and Endophytic Bacteria

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ABSTRACT

Background: Biological control agents are increasingly used as alternatives to chemical methods against plant diseases. This study aimed to identify native bacterial isolates from plant and soil samples capable of suppressing *Rhizoctonia solani* induced root rot in bean (*Phaseolus vulgaris* L.). Given the importance of dry bean production in Turkey, especially in Konya, a key center in Central Anatolia, isolates from this region were prioritized.

Methods: A total of 175 bacterial isolates were collected from rhizosphere and various tissues of beans and *Datura* spp. Their antagonistic activity against *R. solani* AG-4 was assessed *via in vitro* dual culture assays and *in vivo* experiments under controlled conditions using a Randomized Complete Block Design (RCBD). Identification of effective isolates was performed using 16S rRNA gene sequencing.

Result: Ten isolates strongly inhibited fungal growth *in vitro*, with endophytes *Bacillus pumilus* DP25 and *B. velezensis* DP98 showing complete (100%) suppression. *In vivo*, *B. pumilus* DP25 (87%), *B. halotolerans* DP48 (82%) and *B. amyloliquefaciens* DP76 (80%) significantly reduced disease severity; other isolates showed 53-78% efficacy. The study highlights the potential of native *Bacillus* spp. as biocontrol agents, offering practical insights for sustainable bean cultivation in Turkey.

Key words: Biocontrol, Endophytic, *Phaseolus vulgaris*, *Rhizoctonia solani*, Rhizospheric.

INTRODUCTION

Beans (*Phaseolus vulgaris* L.) are nutritionally valuable crops consumed fresh, dried, or canned. However, various pathogens, especially the soil-borne *Rhizoctonia solani* Kühn, which causes root rot, significantly limit production (Meziadi *et al*., 2016). This widespread disease leads to 20-40% global yield losses (Vural and Soylu, 2012; Bhamra *et al*., 2022; Sulaiman and Bello, 2024) and is difficult to control due to the long-term survival of its reproductive structures in the soil. Control strategies include crop rotation, resistant cultivars and primarily fungicides. Although resistant varieties are most effective, their development is time-consuming and new pathogen strains may overcome resistance genes (Palacıoğlu *et al*., 2019).

The primary strategy against these diseases is the use of agricultural pesticides. Although effective in reducing *R. solani*-related symptoms, they do not eliminate sclerotia. Due to concerns about environmental and human health impacts and resistance development, many active ingredients have been banned or increasingly restricted worldwide. Microbial activity also influences the spread of soil-borne diseases. However, natural beneficial microorganisms may not exist in sufficient quantities. Therefore, microbial fertilizers and biopreparations are used to boost disease-suppressing microbes in the soil. Consequently, interest in biological control as an alternative approach has grown (Patel and Singh, 2021).

Biological methods, particularly those utilizing antagonistic interactions of soil- or plant-associated microorganisms, are increasingly favored in plant disease prevention. Plant growth-promoting (PGP) bacteria have proven effective against soil-borne diseases (Bozic *et al*., 2014). These bacteria colonize both the rhizosphere and internal plant tissues as endophytes, residing within sterilized tissues without harming the host. They inhabit various organs such as roots, stems, leaves, seeds, fruits and xylem vessels. Rhizospheric and endophytic bacteria offer eco-friendly disease control while supporting sustainable plant growth (Adeleke *et al*., 2021; Abbas *et al*., 2024). In recent years, *Pseudomonas*, *Bacillus* and *Trichoderma* species have been widely used as biocontrol

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agents, leading to the development of numerous biopreparations.

Biological control studies highlight the potential of endophyte-containing weeds, especially *Datura stramonium* (Jimsonweed) from the Solanaceae family. Despite its harmful effects on crops, *D. stramonium* is a strong competitor. Its rising interest stems from its resistance mechanisms and endophytic bacteria that may suppress or eliminate pathogens while promoting beneficial plant processes. Endophytic antagonistic bacteria isolated from bean fields and *Datura* species have demonstrated effectiveness against various root diseases (Ben Abdallah *et al*., 2016; Sendi *et al*., 2020).

Beyond their biocontrol effects, certain bacterial species produce hydrogen cyanide (HCN), siderophores, cyclic lipopeptides, indole-3-acetic acid (IAA) and solubilize phosphate (Keshavarz-Tohid *et al*., 2017). Investigating the traits of plant growth-promoting microorganisms aids in developing more sustainable biological control strategies. This study aimed to identify bacterial biocontrol agents from indigenous plant and soil samples as an alternative method to control *Rhizoctonia solani*-induced root rot in beans (*Phaseolus vulgaris* L.), particularly in the Konya region where the disease is prevalent.

MATERIALS AND METHODS

This study was conducted at the laboratories of the Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya, Turkey, during the research period 2021-2023.

Source of the pathogen and inoculum preparation

Rhizoctonia solani (GenBank accession no. OQ526209.1) was obtained from the Mycology Laboratory, Faculty of Agriculture, Selçuk University, Konya. It was isolated from infected bean roots showing typical symptoms in a Konya field (Salman and Boyraz, 2023). For inoculum preparation, fungal isolates from PDA stock cultures were incubated at 28±2°C for 7 days to obtain fresh cultures.

Isolation of endophytic and rhizospheric bacteria

Healthy bean (*Phaseolus vulgaris* L.) and *Datura* spp. plants were collected from the same fields for bacterial isolation. Soil samples from their rhizospheres were taken to study rhizospheric bacteria. Endophytes were isolated from plant tissues (leaves, flowers, roots, stems) following Küsek (2007) for soil bioagents and Sendi *et al*. (2020) for plant tissues. Plant materials were washed, surface-sterilized in 1% NaOCl for 1 minute, rinsed thrice with sterile distilled water, dried on sterile paper and placed on nutrient agar. After 24-48 hours incubation, bacterial colonies were purified by streaking on fresh plates. Pure rhizospheric and endophytic cultures were stored at -20°C in 30% glycerol.

Characterization of bacteria

The Gram reaction of endophytic and rhizospheric bacteria cultured for 48 hours was assessed using the potassium hydroxide (KOH) test. For hypersensitive response (HR) testing, bacterial isolates were infiltrated into tobacco leaves and incubated for 48 hours; HR-inducing samples were removed (Schaad *et al*., 2001). Isolates were stored at -20°C in 30% glycerol. Subsequently, their antagonistic effects against plant diseases were evaluated. Bacteria showing antagonism were tested for phosphate solubilization, hydrogen cyanide (HCN) production, indole-3-acetic acid (IAA) production, siderophore production and ACC deaminase activity. Effective bioagents were selected for *in vivo* trials based on a weighted scoring system.

Determination of antagonistic effects of bacteria

Antifungal activity and antibiosis-based control of *R. solani* by bioagents were evaluated using the dual culture test. A 5 mm *R. solani* disc was placed at the center of PDA plates (antibiotic-free) and bacterial cultures were streaked circularly 3 cm from the center. After 7 days incubation at 27°C, fungal growth in treated plates was measured and compared to controls. The experiment used a randomized complete block design (RCBD) with three replicates per treatment. Inhibition percentage was calculated as follows (Tariq *et al*., 2010):

$$\%\ \text{inhibition} = [1 - (\text{Treatment growth}/\text{Control growth})] \times 100$$

Phosphate solubilization

Bacterial colonies were inoculated on Pikovskaya (PVK) medium containing Tri-Calcium Phosphate (TCP) (Pikovskaya, 1948). After 7 days incubation at 28±2°C, colonies with clear halos were considered phosphate-solubilizing (positive).

Indole acetic acid (IAA) production

IAA synthesis was detected following modified methods of Ambrosini and Passaglia (2017) and Aktan and Soylu (2020). Bacteria (10^8 cells/ml) were cultured in LB medium with 1 g/l L-tryptophan at 30°C, 200 rpm for 3 days. After centrifugation (5000 rpm, 30 min), 1 ml supernatant was mixed with 4 ml Salkowski reagent and two drops of orthophosphoric acid. A pink color after 30 minutes indicated IAA production.

ACC deaminase production

The presence of ACC deaminase was inferred from the ability of isolates to utilize ACC as the sole nitrogen source, following Ambrosini and Passaglia (2017). Bacteria were streaked onto DF medium with and without 50 µl of 0.5 M ACC. After 48-72 hours of incubation at 27°C, growth on ACC-supplemented plates compared to ACC-free controls indicated positive ACC deaminase activity.

Siderophores production

Siderophore activity was assessed using the method of Schwyn and Neilands (1987). Bacterial cultures (10^8 cells/ml) were streaked four times onto Blue-CAS agar. Orange-yellow halos around colonies indicated siderophore production.

Hydrogen cyanide (HCN) production

Bacteria were streaked on Tryptic Soy Agar (4.4 g/l) and incubated at 28°C for 3 days. A single colony was then restreaked onto fresh medium. A filter paper soaked in 0.5% picric acid-2% sodium carbonate was placed on the lid and plates were incubated inverted at 28°C for 3-5 days. A yellow to brownish-orange color change indicated HCN production (Bakker and Schippers, 1987).

Selection of bacteria according to the weighted rating system

Candidate bacterial isolates were evaluated using a weighted rating system (WRS) based on predefined coefficients and their performance in characterization tests. Isolates with the highest scores were considered the most effective (Koçak and Salman, 2023).

Molecular characterization

Isolates identified as effective *via* the weighted rating system were analyzed through 16S rRNA gene sequencing. PCR amplification was performed using universal primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-ACG GCT ACC TTG TTA CGA CTT-3′) (Weisburg *et al*., 1991) with a PTC 100 thermal cycler (M.J. Research, USA). Sequences from 10 isolates were compared with NCBI database entries. Partial 16S rDNA sequences were submitted to GenBank under accession numbers PP125781.1–PP125790.1.

Determination of *in vivo* biocontrol activity

Bacterial isolates effective *in vitro* were further tested *in vivo* against *R. solani*. Bean seeds were surface-sterilized with 2% sodium hypochlorite, rinsed with sterile water and pre-germinated on diluted water agar. Upon sprouting, three seeds were sown per pot containing 2 kg sterilized peat-perlite mix. Bacterial suspensions (10^8 cells/ml) were prepared from 48-hour cultures and seeds were bioprimed by immersion in 15 ml of the suspension for 10 minutes. Barley grains inoculated with *R. solani* and treated seeds were placed in pots, followed by the addition of the remaining bacterial suspension. Root lesions were evaluated using the scale of Muyolo *et al*. (1993): healthy seedlings (0 points); very small, superficial brown lesions on roots or stems (1 point); deep and wide lesions on roots or stems with retardation in root development (2 points); severe root rot with deep lesions surrounding the main root or stem and significantly reduced root length (3 points); and dead plants (4 points). All experiments were performed in triplicate. Biocontrol efficacy was calculated using Abbott's formula:

Efficacy (%) =

$$\frac{\text{Disease in control - Disease in treatment}}{\text{Disease in control}} \times 100$$

Statistical analysis

Disease scale values were analyzed using Minitab 17 and mean differences were evaluated with Fisher's LSD test at P = 0.05.

RESULTS AND DISCUSSION

Identification of selected antagonistic bacteria

Survey studies in eight bean fields yielded 56 samples from soil and healthy plants (*Phaseolus vulgaris* L. and *Datura* spp.), resulting in 175 bacterial isolates 140 from plant tissues and 35 from rhizospheric soil. Among the 10 most bioactive isolates, 9 were endophytic and only one (DP143.6) was rhizospheric.

Dual culture and biochemical test results were evaluated using a weighted rating system with a base score of 70. The top 10 isolates were identified by 16S rRNA gene sequencing and sequences were submitted to NCBI GenBank (Table 1).

BLAST comparisons with GenBank reference sequences confirmed ~100% similarity to *Bacillus* spp. (Fig 1). Phylogenetic analysis revealed that *Bacillus altitudinis* DP26 formed an early-diverging branch, suggesting distinct evolutionary history. *B. pumilus* DP25, *B. aerophilus* DP45 and *B. altitudinis* DP88 clustered in a strongly supported clade (100% bootstrap), indicating close genetic relatedness. Similarly, *B. amyloliquefaciens* DP76 and *B. velezensis* DP98 shared a common ancestor. *B. halotolerans* DP47 and DP48 grouped with *B. subtilis* DP143.6, also with 100% bootstrap support, indicating close genetic affiliation.

In vitro dual culture antagonism

Fifty-three bacterial isolates showed efficacy against *Rhizoctonia solani*. Some isolates completely inhibited mycelial growth, while others partially controlled it. Among these, 29 exhibited over 70% antagonism and four (DP25, DP64, DP81, DP98) achieved 100% inhibition. The remaining isolates showed 62–70% inhibition. Forty-two isolates were endophytic and the rest were rhizospheric. Dual culture assessed antagonistic activity, complemented by biochemical (PGP) traits. The top candidates were selected *via* a weighted grading system and tested on host plants.

The weighted evaluation results in Fig 2 and Table 2 reveal significant differences among the 10 bacterial isolates in suppressing *R. solani*.

Biochemical properties of bacterial antagonists

Growth-promoting potential (PGP) of 53 biocontrol isolates was assessed using a standard curve, revealing that 81% produced IAA between 2.2-13.2 ppm; all but DP76 and DP78 showed strong IAA activity. Except for 13 isolates, all tested positive for siderophore production (2-32 mm). While 30 isolates solubilized phosphorus, only DP78, also selected *in vivo*, was negative. Among four ACC-positive isolates, DP48 used in the next study phase tested positive. Only seven isolates produced HCN, including DP78. Ten isolates with the highest activity were selected for *in vivo* tests (Table 3). Biochemical and dual culture results were scored *via* a weighted rating system with a base of 70 points.

Ability of bacterial isolates to control the pathogen *in vivo*

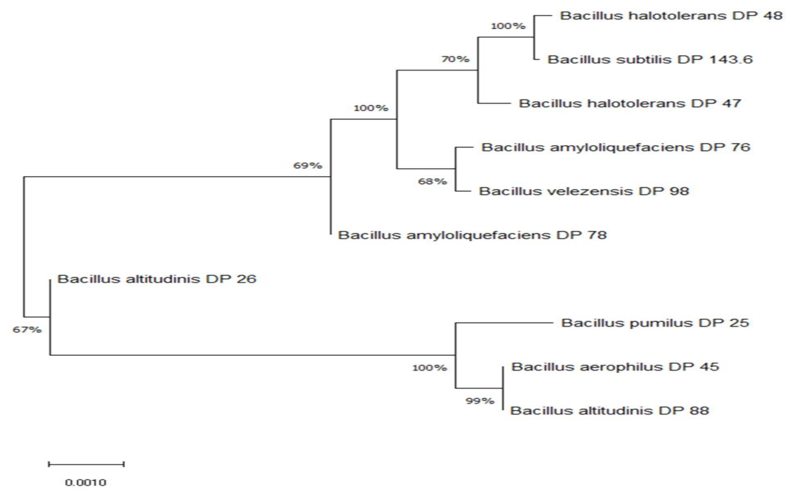
Under climate chamber conditions, the ability of bacterial isolates to control *Rhizoctonia solani* induced root rot in beans was evaluated. All isolates significantly ($P<0.05$) reduced disease severity compared to the negative control (Table 4, Fig 3).

Biocontrol efficacy was calculated by comparing treated plants to the positive control and is presented in Table 4. Except for *Bacillus amyloliquefaciens* DP88, nine isolates showed over 50% efficacy *in vivo*. *Bacillus pumilus* DP25 showed the highest control (87%), followed by *B. halotolerans* DP48 (82%) and *B. amyloliquefaciens* DP76 (80%). *Bacillus amyloliquefaciens* DP78, *B. altitudinis* DP26 and *B. subtilis* DP143.6 exhibited 73-76% efficacy, while *B. halotolerans* DP47 and *B. velezensis* DP98 had similar inhibition (~78%). Except for *B. aerophilus* DP45 (53%) and *B. amyloliquefaciens* DP88 (40%), eight isolates demonstrated strong antagonistic potential as biocontrol agents against bean root rot. Fig 3 shows the effectiveness of bacterial bioagents at a high efficacy against *R. solani in vivo*.

R. solani survives in soil for years due to its sclerotia, making control difficult; thus, chemical methods are often preferred (Liu *et al*., 2021). However, ecological alternatives using chemical-free products are increasingly recommended to avoid harmful effects on human health and the environment. Many countries market microorganisms with biocontrol activity, known as biopesticides (Abbas *et al*., 2019). This study evaluated the traits and biocontrol

Table 1: Molecular identification of bacterial isolates using the 16S rRNA subunit and scoring them against *Rhizoctonia solani* according to weighted grading.

Molecular Identification			
Bacterial isolates	Blast results	Accession number	Weighted grading
DP25	*Bacillus pumilus*	PP125781.1	70
DP26	*Bacillus altitudinis*	PP125787.1	70
DP45	*Bacillus aerophilus*	PP125789.1	70
DP47	*Bacillus halotolerans*	PP125784.1	70
DP48	*Bacillus halotolerans*	PP125782.1	90
DP76	*Bacillus amyloliquefaciens*	PP125783.1	70
DP78	*Bacillus amyloliquefaciens*	PP125786.1	70
DP88	*Bacillus amyloliquefaciens*	PP125790.1	70
DP98	*Bacillus velezensis*	PP125785.1	70
DP143.6	*Bacillus subtilis*	PP125788.1	70



The phylogenetic tree was constructed using the Maximum Composite Likelihood method in MEGA X (Saitou and Nei, 1987; Felsenstein, 1985; Tamura *et al*., 2004). Tree robustness was evaluated with 1000 bootstrap replicates (Kumar *et al*., 2018).

Fig 1: Neighbor-joining phylogenetic tree of selected bacterial 16S rRNA sequences antagonistic to *Rhizoctonia solani* OQ526209.1.

potential of naturally occurring endophytic and rhizospheric bacteria against *R. solani* induced bean root rot. Unlike previous research focusing solely on rhizobacteria, isolating both bacterial types from the same ecosystem allows comparative analysis of their biocontrol mechanisms.

The *Bacillus* genus, widely used as beneficial biopesticides, included many species isolated in this study. Previous research has demonstrated *Bacillus* potential as biological control agents against *R. solani*. In the dual culture test, 32 isolates significantly (over 70%) inhibited *R. solani* mycelial growth, consistent with earlier studies using bacterial biocontrol agents against this pathogen (Aydın, 2022; Lan *et al*., 2024).

Bacillus species exhibit diverse antifungal mechanisms, producing low molecular weight antifungal compounds, especially lipopeptide antibiotics (bacillomycin, fengycin, surfactin) and bacteriocins critical for biocontrol (Radhakrishnan *et al*., 2017). Our study indicates that *B. subtilis* isolates with high inhibition likely produce these lipopeptides, as supported by Mnif and Ghribi (2015). Biocontrol agents with lipopeptide genes have been commercialized due to their enhanced fungal suppression and antibiotic production (Joshi and McSpadden Gardener, 2006). Recent genomics reveal that gene copy number variations, such as ituD, affect lipopeptide synthesis and biocontrol efficacy (Wang *et al*., 2024; Bai *et al*., 2025). Genetic analysis of our top isolates could clarify their mechanisms.

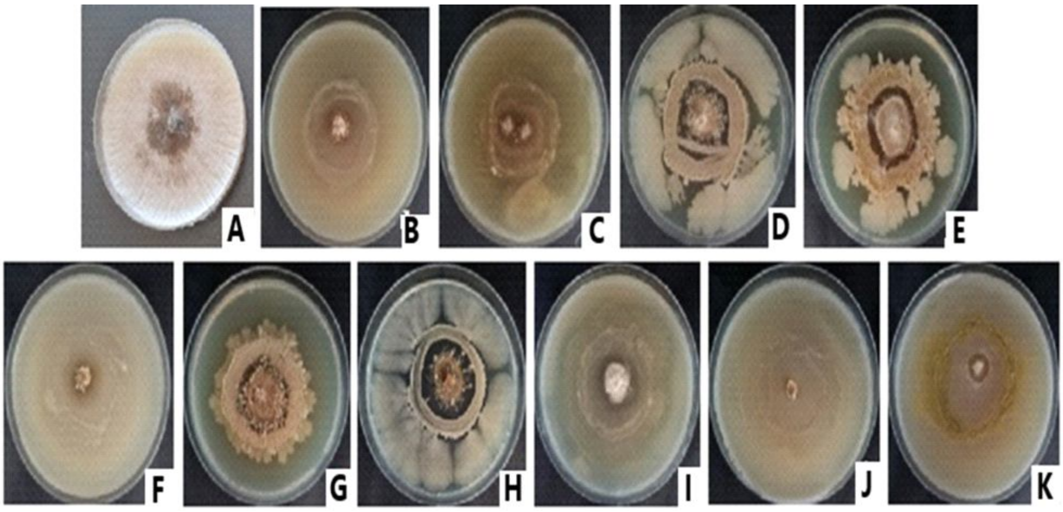
Endophytic *Bacillus* species have gained attention for their positive impact on plant health. Fall *et al*. (2004) reported root colonization by *B. subtilis*. In this study, endophytic *B. subtilis* strains showing 73% inhibition underscore their dual biocontrol potential in both rhizosphere and plant tissues.

Martins *et al*. (2018) similarly confirmed that 76-80% of isolated *B. amyloliquefaciens* strains function as effective

Table 2: Evaluation of the inhibitory effect of selected *Bacillus* species against *Rhizoctonia solani.*

Bacterial Isolate	Treatment group diameter (cm)	Inhibition rate (%)
Bacillus pumilus (DP25)	0	100a
Bacillus velezensis (DP98)	0	100a
Bacillus aerophilus (DP45)	1.08	88ab
Bacillus halotolerans (DP47)	1.08	88ab
Bacillus halotolerans (DP48)	1.35	85ab
Bacillus amyloliquefaciens (DP88)	1.71	81b
Bacillus amyloliquefaciens (DP78)	1.89	79b
Bacillus subtilis (DP143.6)	2.34	74b
Bacillus amyloliquefaciens (DP76)	2.61	71b
Bacillus altitudinis (DP26)	2.61	71b
R. solani (Control)	9.00	0c

$P<0.05$ (The means represented by the same letter within the same column do not differ statistically).



(A) Control; (B) *B. pumilus* DP25; (C) *B. altitudinis* DP26; (D) *B. aerophilus* DP45; (E) *B. halotolerans* DP47; (F) *B. halotolerans* DP48; (G) *B. amyloliquefaciens* DP76; (H) *B. amyloliquefaciens* DP78; (I) *B. amyloliquefaciens* DP88; (J) *B. velezensis* DP98; (K) *B. subtilis* DP143.6.

Fig 2: Antagonistic activity of *Bacillus* isolates against *R. solani*.

biocontrol agents. Our findings further support the hypothesis that the tested isolates suppress pathogens primarily through antibiosis.

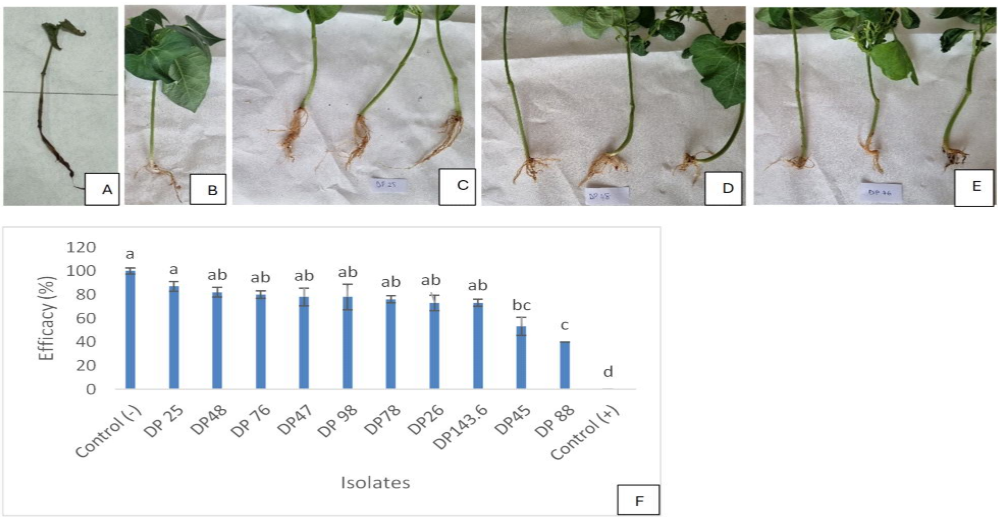
Endophytic bacteria appear more prominent in the plant microbiome than rhizospheric ones, as 99% of the isolates effective against *R. solani* in this study were endophytes. They offer stronger protection against environmental stress (Miliute *et al*., 2015). Unlike rhizobacteria, which mainly promote growth, endophytes form stable, direct interactions with plants, ensuring more consistent and lasting protection.

Bacillus species are among the most important plant growth-promoting (PGP) bacteria, capable of producing phytohormone-like compounds that stimulate plant growth. Various species, including *B. megaterium, B. thuringiensis, B. pumilus, B. weihenstephanensis, B. cereus, B. toyonensis and B. subtilis,* have been isolated from rhizospheric and endophytic environments and reported to produce indole-3-acetic acid (IAA) (Martins *et al*., 2018; Shabanaand Ambreen, 2019). Studies suggest that approximately 80% of plant-associated bacteria are capable of IAA production; for instance, 81% of 61 isolates from the potato rhizosphere were shown to produce IAA (Calvo *et al*., 2010). However, the amount of IAA produced varies among isolates. For example, endophytic and rhizospheric bacteria isolated from wheat have been reported to produce IAA in the range of 0.27 to 77.98 ppm (Majeed *et al*., 2015). In our study, 64.2% of the tested isolates produced IAA within the range of 1.4 to 13.2 ppm. These findings align with those of Elsoud *et al*. (2023), who reported significant *in vitro* IAA production by *Bacillus* species. However, it is important to note that *in vitro* IAA production levels (1.4-13.2 ppm) may not directly reflect field conditions. Boonmahome and

Table 3: The plant growth-promoting activities of the selected 10 antagonistic bacterial isolates.

Isolate	IAA production (ppm)	ACC deaminase	Siderophore production (mm)	Inorganic phosphate solubilization	HCN production
DP25	7.3 (+)	-	4 (+)	+	-
DP26	8.5 (+)	-	20 (+)	+	-
DP45	6.8 (+)	-	8 (+)	+	-
DP47	7.1 (+)	-	14 (+)	+	-
DP48	7.3 (+)	+	14 (+)	+	-
DP76	2.8 (+)	-	13 (+)	+	-
DP78	3.7 (+)	-	25 (+)	-	+
DP88	7.7 (+)	-	2 (+)	+	-
DP98	6.9 (+)	-	20 (+)	+	-
DP143.6	7.1 (+)	-	11 (+)	+	-

The presence of an activity is indicated by '+', and the absence is indicated by '-'.



(A) Control (+); (B) Control (-); (C) *Bacillus pumilus* (DP25); (D) *Bacillus halotolerans* (DP48); (E) *Bacillus amyloliquefaciens* (DP76) antifungal activity and efficacy (%) and (F) graphical efficacy (%) of bacterial isolates against the growth of *Rhizoctonia solani*.

Fig 3: The effect of bacterial isolates on the control of *Rhizoctonia solani* on bean plants.

Table 4: The efficacy of bacterial isolates in the biological control of *Rhizoctonia solani.*

Bacterial species	Disease severity (%)	Efficacy (%)
Bacillus pumilus (DP 25)	13±2.64[d]	87±2.64[a]
Bacillus halotolerans (DP48)	18±4.16[d]	82±4.16[a]
Bacillus amyloliquefaciens (DP 76)	20±4.00[cd]	80±4.00[ab]
Bacillus halotolerans (DP47)	22±3.00[cd]	78±3.00[ab]
Bacillus velezensis (DP 98)	22±7.37[cd]	78±7.37[ab]
Bacillus amyloliquefaciens (DP78)	24±10.69[cd]	76±10.69[ab]
Bacillus altitudinis (DP26)	27±3.05[cd]	73±3.05[ab]
Bacillus subtilis (DP143.6)	27±6.43[cd]	73±6.43[ab]
Bacillus aerophilus (DP45)	47±3.00[bc]	53±3.00[bc]
*Bacillus amyloliquefaciens (*DP 88)	60±7.63[b]	40±7.63[c]
Control (-)	0±0.00[d]	100±0.00[a]
Control (+)	100±0.00[a]	0±0.00[d]

P<0.05 (The means represented by the same letter within the same column do not differ statistically).

Mongkolthanaruk (2023) demonstrated that IAA stability decreases by up to 70% when soil pH falls below 6.5 and temperature drops.

Positive phosphate-solubilizing activity was observed in 45% of the tested isolates. Phosphate-solubilizing bacteria enhance the availability of phosphorus to plants by mineralizing organic phosphorus compounds and converting inorganic phosphorus into more usable forms. The acids produced by these bacteria lower the pH, releasing phosphorus trapped in calcareous soils and contributing to biological disease control (Rasul *et al*., 2019). Phosphatase activity was detected in 15% of the 499 potential PGP bacterial isolates obtained from 39 different soil samples (Çetinkaya Yildiz and Aysan, 2014). Furthermore, many studies have shown that endophytic and rhizospheric bacteria possess phosphate-solubilizing capabilities (Ahmad *et al*., 2008; Özaktan *et al*., 2015).

Hydrogen cyanide (HCN), a broad-spectrum antibacterial agent, is used in biocontrol of root diseases by inhibiting cytochrome oxidase in the respiratory chain, thereby disrupting pathogen energy synthesis. Our study found that the tested bacteria produced notable levels of HCN. While HCN is recognized as a key secondary metabolite in biocontrol (Nandi *et al*., 2017), its role in suppressing pathogenic fungi remains debated, likely due to differing experimental conditions (Michelsen and Stougaard, 2012).

Siderophore production is an effective strategy employed by bacterial biocontrol agents (Kesaulya *et al*., 2018). In particular, *Bacillus* species synthesize siderophores that chelate and solubilize iron, thereby promoting plant growth. Additionally, they inhibit the growth of phytopathogenic bacteria and fungi, while supporting water movement and ionic balance in plant tissues (Joshi and McSpadden Gardener, 2006). In this study, 85% of the isolates were capable of producing siderophores, forming halos ranging from 2 to 32 mm in diameter. *Bacillus amyloliquefaciens* DP78 exhibited a high antagonistic potential with a 25 mm halo. Priyanka Agrawal *et al*. (2017) reported a strong correlation between siderophore production and antifungal activity.

Furthermore, plant growth-promoting (PGP) bacteria are known to utilize 1-aminocyclopropane-1-carboxylate (ACC) as a nitrogen source by converting it into ACC deaminase (ACC-d), which enhances plant growth (Gupta and Pandey, 2019). However, only 7.6% of the isolates in this study demonstrated ACC-d activity.

The top ten isolates were identified by 16S rRNA sequencing following characterization and dual-culture tests. These included *B. pumilus* DP25, *B. halotolerans* DP48, *B. amyloliquefaciens* DP76, DP78 and others. Numerous studies have demonstrated the effectiveness of *Bacillus* species as biocontrol agents against *R. solani in vitro* and *in vivo* (Sunkad *et al*., 2023; Sirivella *et al*., 2025).

In vivo biocontrol tests provided a clearer evaluation of the isolates' antagonistic potential, highlighting that *in vitro* and *in vivo* results do not always correlate. Notably, *B. amyloliquefaciens* DP88 showed strong *in vitro* antagonism but only 40% disease inhibition *in vivo.* Three isolates *B. pumilus* DP25, *B. halotolerans* DP48 and *B. amyloliquefaciens* DP76 exceeded 80% efficacy. Others *(B. halotolerans* DP47, *B. velezensis* DP98, *B. amyloliquefaciens* DP78, *B. altitudinis* DP26, *B. subtilis* DP143.6 and *B. aerophilus* DP45) inhibited *R. solani* between 53% and 78%.

Our study identified *B. amyloliquefaciens* DP76, *B. halotolerans* DP48 and *B. pumilus* DP25 as the most effective biocontrol agents against *R. solani*. These findings support previous research (Feng *et al*., 2022; Abdelaziz *et al*., 2023; Kumar *et al*., 2023; Resmi *et al*., 2024) and offer new strategies for sustainable agriculture.

CONCLUSION

The results indicate that both plant tissues and soil may serve as reservoirs for bacteria with diverse PGP traits and antagonistic activity. Due to their endospore-forming ability, *Bacillus* isolates can persist under varying environmental conditions. Therefore, the tested isolates hold potential as antagonistic PGP agents against *R. solani* in bean cultivation. However, field trials are needed to validate their biocontrol and growth-promoting effects. Most endophytic and rhizospheric bacteria identified in this study show promise as biocontrol agents in commercial bean production and may also enhance soil health, nutrient availability and microbial balance, contributing to improved crop yield.

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Disclaimers

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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