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RESEARCHARTICLE

Trends in Biotechnology and Plant Sciences

Chitinolytic Rhizobacteria for Sustainable Management of Soybean Stem Rot Caused by Rhizoctonia solani

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Abstract

Stem rot caused by *Rhizoctonia solani* is a destructive disease that threatens soybean (*Glycine max*) production worldwide. Environmentally sustainable control strategies are urgently needed to replace or complement fungicides, which pose environmental risks and promote resistance. This study evaluated ten chitinolytic bacterial isolates for their ability to suppress *R. solani* and promote soybean growth. In vitro dual culture assays showed that five isolates (ST21e, SS12b, ST17c, ST27d, and ST26c) exhibited high antagonism, with inhibition rates exceeding 40%. Greenhouse trials revealed that bacterial treatments significantly reduced stem rot incidence compared with the untreated control, which reached 67.5% by week six. Notably, isolates ST27d and ST17c completely prevented disease symptoms throughout the observation period. In addition, these isolates enhanced soybean performance, with ST27d producing the tallest plants (75.28 cm) and the highest leaf number (32 trifoliates) at the final observation. The results confirm that selected chitinolytic bacteria offer dual benefits of disease suppression and plant growth promotion, underscoring their potential as bio-based alternatives for soybean stem rot management. These findings provide a strong foundation for further development of microbial inoculants to support sustainable soybean cultivation.

KEYWORDS
soybean, *Rhizoctonia solani*, stem rot, chitinolytic bacteria, biological control, plant growth promotion, sustainable agriculture.

1 | INTRODUCTION

Soybean (*Glycine max*) is one of the most important legume crops globally, contributing substantially to protein and oil demands in human and animal nutrition. However, this crop's productivity is often compromised by soil-borne pathogens. Among them, *Rhizoctonia solani* is notorious for causing stem rot, damping-off, basal lesions, and yield reductions in soybean (Haque et al., 2024). Given its ability to survive in soil and cause disease even under favorable moisture conditions, control of *R. solani* remains challenging, especially for resource-limited farmers (Nowak et al., 2025; Ali et al., 2025).

Traditionally, chemical fungicides have served as the go-to strategy for managing *R. solani* in soybean and other crops. However, repeated fungicide use raises multiple concerns: development of resistant fungal strains, negative impacts on non-target organisms and soil health, environmental contamination, and increased production costs (Haqq, 2024; Ali et al., 2025). These drawbacks have driven research towards more sustainable, eco-friendly alternatives that provide effective disease suppression with minimal side effects.

One promising approach is the use of plant growth-promoting rhizobacteria (PGPR) that produce chitinases—enzymes that degrade chitin, a key component of fungal cell walls. Chitinolytic bacteria attack the structural integrity of pathogens like *R. solani*, thereby inhibiting their growth (Abo-Zaid et al., 2024; Shams et al., 2025). Beyond antagonism, many of these bacteria offer dual benefits: they enhance plant growth through mechanisms such as phosphate solubilization, siderophore production, hormonal modulation, or improved nutrient uptake (Solanki et al., 2022; Nowak et al., 2025).

Recent studies have advanced our understanding of these dual functions. For example, Solanki, Khan, and colleagues (2022) demonstrated that antagonistic *Pseudomonas* and *Bacillus* strains both reduced *R. solani* severity in soybean and increased soil dehydrogenase, chitinase, and glucanase enzyme activities. These changes in enzyme activities were correlated with lower pathogen load and improved plant vigour (Solanki et al., 2022). Similarly, Nowak et al. (2025) reported that *Priestia megaterium* strain KW16 produced chitinases, siderophores, volatile organic compounds, and other PGPR traits; in plants challenged with *R. solani*, KW16 significantly increased shoot and root biomass compared to pathogen-only controls (Nowak et al., 2025).

In another recent work, Abo-Zaid et al. (2024) isolated *Streptomyces cellulosae* Actino 48, a chitinase-producing actinobacterium, which inhibited over 97% of *R. solani* mycelial growth in vitro. Under greenhouse conditions, bio-formulations of this isolate reduced disease incidence (damping-off and root rot) in peanut plants and significantly improved plant survival and biomass (Abo-Zaid et al., 2024). Shams et al. (2025) also reported that *Streptomyces lividans* and *S. rochei* strains suppressed *R. solani* in vitro and improved health and survival of green beans under pathogen pressure (Shams et al., 2025).

Despite these promising advances, there remains a gap in the literature when it comes to *soybean stem rot* specifically managed by chitinolytic rhizobacteria. Many studies have focused on other crops (peanut, green bean, etc.), or on other forms of disease (root rot, damping-off), leaving a need for trials under conditions that mimic soybean field/environmental stresses (Haqq, 2024; Hussein et al., 2024). Moreover, while some PGPR strains have been identified, comprehensive evaluation combining in vitro antagonism, chitinase activity, greenhouse disease suppression, and plant growth promotion metrics is less common.

The molecular responses of soybean itself also play a part. Recent characterization of soybean chitinase genes (e.g., *GmChi01, GmChi02, GmChi16*) showed that overexpression of *GmChi02* or *GmChi16* in Arabidopsis conferred improved resistance against *Fusarium oxysporum*. These findings suggest that enhancing chitinase activity in soybean (either via host genetics or through induction by chitinolytic bacteria) could be a useful route for disease resistance (Chen et al., 2024). However, cross-pathogen applicability (i.e. for *R. solani*) and field-scale relevance remain to be tested.

In addition, the soil microenvironment and rhizosphere microbial community are emerging as important modulators of disease suppression. PGPR may influence microbial community structure, boosting beneficial organisms and reducing pathogen prevalence, thereby contributing to long-term suppressiveness of soil (Solanki et al., 2022; Ali et al., 2025). Also, optimized formulations (e.g., using carriers, nano or talc formulations) are shown to enhance persistence and practical usability of biocontrol agents (Abo-Zaid et al., 2024).

Given all of this, our study seeks to address the following central questions: Can indigenous chitinolytic rhizobacteria from the soybean rhizosphere effectively suppress stem rot disease caused by *R. solani* under greenhouse conditions? Do those same isolates also contribute measurably to soybean growth and health?

Therefore, the objectives of this research are three-fold: (1) to isolate and screen chitinolytic rhizobacteria for antagonism against *R. solani* and high chitinase enzyme activity; (2) to evaluate their effectiveness in suppressing soybean stem rot under controlled greenhouse evaluations; (3) to assess their plant growth promotion effects (root/shoot biomass, seedling vigour) and explore any shifts in rhizosphere health indicators (e.g., soil enzyme activities, microbial community proxies). By integrating pathogen suppression with growth promotion, this study aims to contribute to sustainable disease management strategies that are practical, ecologically sound, and suitable for soybean farmers contending with *R. solani*.

2 MATERIALS AND METHODS

Ten isolates of chitinolytic bacteria previously characterized for their ability to degrade chitin were used in this study, along with two isolates of *Rhizoctonia solani* obtained from diseased soybean plants. The bacterial isolates differed in soil origin, Gram reaction, ability to fluoresce under UV light, and the diameter of the clear zone produced on chitin-containing media. Their characteristics are presented in Table 1. The two *R. solani* isolates were used as test pathogens in both in vitro and in planta assays.

Table 1: Characteristics of chitinolytic bacterial isolates used in this study

Isolate Code	Soil pH	Gram Reaction	Fluorescence (UV)	Chitinolytic Zone Diameter (cm)
ST06d	4.1	–	–	2.2
ST17c	5.4	–	–	3.4
ST21b	5.8	–	–	2.6
ST21e	5.8	+	–	4.6
ST26c	5.9	–	–	4.6
ST27d	4.6	–	–	2.8
SS01b	5.7	–	–	2.8
SS07c	5.4	–	–	2.2
SS12b	5.3	–	+	2.2
SS17b	4.6	–	–	2.2

"+" indicates positive reaction; "–" indicates negative reaction.

The antagonistic effect of the chitinolytic bacteria against *R. solani* was first assessed under in vitro conditions. Bacterial isolates were cultured on tryptic soy

agar for 48 hours at room temperature. Each isolate was then streaked along the margin of a potato dextrose agar (PDA) plate, leaving a distance of 3 cm from the plate edge. After 24 hours of bacterial growth, a 0.5 cm plug of actively growing *R. solani* mycelium was placed on the opposite side of the plate at a distance of 3 cm from the bacterial colony. Plates were incubated at room temperature for six days. Fungal growth was measured in two directions: R1, the radius of mycelial growth toward the plate margin, and R2, the radius of growth toward the bacterial colony. The inhibition rate was calculated as:

$$\text{Inhibition Rate} = \frac{R1 - R2}{R1} \times 100\%$$

Each bacterial isolate was tested against both pathogen isolates in duplicate.

To evaluate the potential of selected chitinolytic bacteria as biocontrol agents under greenhouse conditions, a completely randomized design was employed with six treatments consisting of five bacterial isolates (ST21e, SS12b, ST17c, ST27d, and ST26c) and a non-inoculated control. All treatments except the control were challenged with *R. solani*. Each treatment unit consisted of ten soybean plants, and each treatment was replicated four times, resulting in a total of 240 plants.

The growth medium was prepared from Ultisol soil mixed with cattle manure in a ratio of 3:1 (w/w). The mixture was sterilized by autoclaving at 121 °C and 1 atm pressure and dispensed into plastic pots, each containing 2 kg of soil. Pathogen inoculum was produced by culturing *R. solani* in potato dextrose broth for 14 days. The mycelia were harvested, rinsed, and suspended in sterile water. This suspension was then mixed with sterilized sand at a ratio of 1:5 (w/v) to prepare inoculum. The sand–mycelium mixture was incorporated into the potting soil at a rate of 10 g per pot, after which the soil was incubated for seven days prior to sowing.

Soybean seeds were surface-sterilized and soaked in bacterial suspensions (10^7 cfu mL^{-1}) for six hours. Seeds were air-dried under sterile conditions and sown into the prepared pots. At 14 days after sowing, a second application of bacterial suspension (10 mL per plant at 10^7 cfu mL^{-1}) was administered by drenching around the root zone.

Data were collected weekly from two to six weeks after sowing. Parameters measured included disease incidence (percentage of infected plants), plant height, and number of trifoliate leaves per plant. Data were subjected to analysis of variance, and mean differences were tested using Duncan's multiple range test at a 5% probability level.

3 RESULTS

The antagonism assay demonstrated that all chitinolytic bacterial isolates tested were capable of inhibiting the growth of *Rhizoctonia solani* in vitro. However, the magnitude of inhibition varied among isolates. Five isolates (ST21e, SS12b, ST17c, ST27d, and ST26c) consistently exhibited strong antagonistic activity, with average inhibition values above 40%. The isolate ST21e showed the highest inhibition rate (49.16%), followed closely by SS12b (45.83%) and ST17c (45.00%). Isolates ST27d and ST26c also maintained substantial inhibitory effects of 44.16% and 42.50%, respectively. By contrast, the remaining isolates displayed weaker inhibition, ranging between 21.67% and 26.67% (Figure 1). These results confirmed the capacity of selected isolates to limit fungal growth and justified their subsequent use in greenhouse evaluation.

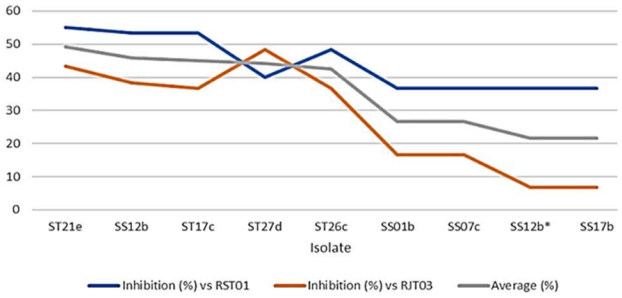

Fig. 1. In vitro inhibition (%) of chitinolytic bacterial isolates against *Rhizoctonia solani* six days after inoculation. *Note: duplicate entry SS12b corresponds to a weakly antagonistic isolate from a separate sampling site.

Greenhouse trials revealed a marked reduction in stem rot incidence following seed and seedling treatment with chitinolytic bacteria. The untreated control exhibited severe disease symptoms, with incidence reaching 67.5% by the sixth week. In contrast, plants treated with isolates ST27d and ST17c remained completely free of stem rot throughout the observation period. Other isolates also reduced disease incidence, although to varying degrees. For example, SS12b and ST21e restricted disease progression during the early weeks but showed some infection at later stages. Overall, the results demonstrated that all tested isolates reduced disease compared with the control, with ST27d and ST17c providing the most consistent protection (Figure 2).

Plant growth parameters also responded positively to bacterial application. Soybean plants treated with ST27d consistently displayed the greatest plant height at all observation periods, significantly surpassing other treatments. By the sixth week, plants in this treatment reached an average height of 75.28 cm, followed by ST17c (48.99 cm). Other isolates also improved plant

height compared with the control, which averaged only 17.45 cm at the final observation (Figure 3).

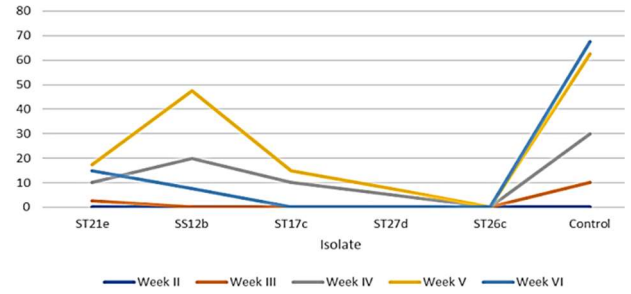

Fig. 2: Effect of chitinolytic bacterial treatments on incidence of soybean stem rot caused by *Rhizoctonia solani* (%) at weekly observations.

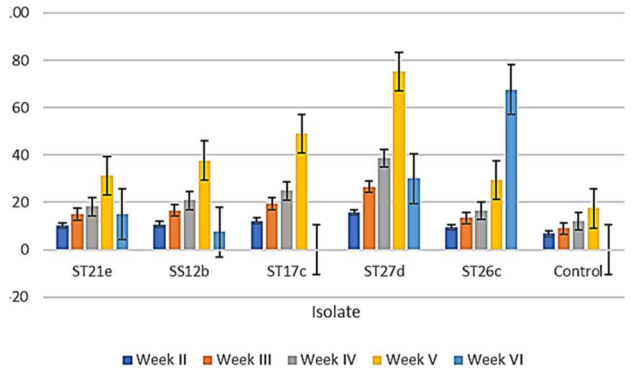

Fig. 3: Effect of chitinolytic bacterial treatments on soybean plant height (cm) at weekly observations.

Leaf production was similarly enhanced by bacterial application. Treatments with ST27d and ST17c produced the greatest number of trifoliate leaves, with 32 and 24 leaves per plant, respectively, at the sixth week. These values were significantly higher than the control, which produced only 13 leaves during the same period. The other isolates also contributed to leaf production but with intermediate results between the high-performing isolates and the untreated control (Figure 4).

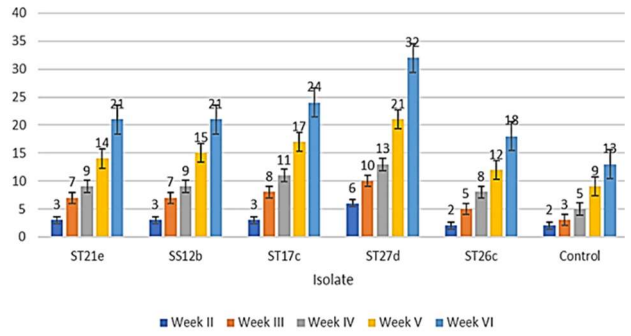

Fig. 4: Effect of chitinolytic bacterial treatments on the number of trifoliate leaves of soybean plants at weekly observations.

Overall, these results confirmed that several chitinolytic bacterial isolates were effective not only in suppressing stem rot caused by *R. solani* but also in promoting plant growth. Among them, isolates ST27d and ST17c consistently provided the strongest protection against disease and contributed significantly to improved height and leaf production.

4 | DISCUSSION

The results of this study revealed that the chitinolytic bacterial isolates ST27d and ST17c performed exceptionally well as biocontrol agents against *Rhizoctonia solani*, both in vitro and in planta, while also promoting soybean growth in height and foliage. These findings align with emerging trends in PGPR research, which emphasize obtaining dual benefits: disease suppression combined with growth promotion (Ehinmitan et al., 2024; Chaudhary et al., 2024). Below, the discussion unpacks possible mechanisms, compares with recent related work, identifies limitations, and suggests future research directions.

The high inhibition percentages of ST21e, ST17c, ST27d, and ST26c in vitro (Figure 1) suggest that the chitinolytic bacteria employed active antagonistic mechanisms against *R. solani*. Such mechanisms likely involve chitinase enzyme secretion, but could also include production of volatile organic compounds (VOCs), siderophores, and other antifungal metabolites. Similar multi-trait action has been documented in *Priestia megaterium* KW16, which strongly inhibited *R. solani* growth via VOCs and secreted enzymes while also enhancing biomass under pathogen pressure (Nowak et al., 2025). Likewise, Shams et al. (2025) reported *Streptomyces lividans* and *S. rochei* strains having high in vitro suppression of *R. solani*, including via lytic enzymes and culture filtrate effects. These parallels support the hypothesis that high in vitro antagonism often arises from multiple, complementary antifungal strategies.

In planta, the dramatic reduction of disease incidence in treatments with ST27d and ST17c (even to zero in several observations) demonstrates not merely antagonism in controlled petri dishes but effective expression of biocontrol in more complex living systems. This is crucial: field-or greenhouse-scale effects often diverge from in vitro results due to soil interactions, plant immune responses, microbial competition, and inoculum persistence (Etesami, 2024; Chaudhary et al., 2024). For instance, in a study of integrated biocontrol and fungicide use in soybean, *Pseudomonas fluorescens* showed around 43% inhibition of *R. solani* in vitro and, when used with a compatible fungicide, reduced disease incidence by approximately 75% and improved growth and photosynthetic traits under greenhouse conditions

(Enhancing Biotic Stress Tolerance, 2023). While our work did not use a chemical fungicide component, the near-complete suppression by ST27d and ST17c approaches that level of efficacy and shows promise for pathogen control without chemical inputs.

Growth promotion by those isolates was also marked: at week six the ST27d treatment achieved the greatest height and leaf count, followed by ST17c, significantly beyond control plants. These findings are in line with documented PGPR traits in recent literature. Ehinmitan et al. (2024) in their review highlighted how PGPR strains may improve nutrient availability (e.g. phosphate solubilization), hormone modulation, and root growth which collectively improve above-ground growth. In a study of microbial consortia by *Indigenous bacterial consortia for growth promotion…* (2025), similar improvements in soybean biomass were observed when growth-promoting traits were strong. Also, *Priestia megaterium* KW16 had over 200% increase in shoot biomass under pathogen challenge (Nowak et al., 2025). Thus the growth promotion seen with ST27d and ST17c is consistent with known PGPR function.

Mechanistically, chitinases are central. By degrading chitin in fungal cell walls, chitinolytic bacteria weaken pathogen structure, reducing the ability to colonize plant roots or stems. In addition, cell wall degradation may release elicitor fragments that trigger plant immune responses (induced systemic resistance, ISR). A recent comprehensive review on microbial bio-control agents (Chaudhary et al., 2024) emphasized that isolates encoding chitinases, glucanases, and other cell wall–degrading enzymes are among the most reliable in suppressing soil-borne fungi. Furthermore, KW16 genome analysis provided evidence that expression of genes for lytic enzymes, siderophores, phytohormones, and VOCs all act in concert (Nowak et al., 2025). It is plausible that ST27d and ST17c similarly combine multiple mechanisms, enabling strong antagonism and promoting growth.

Our results also suggest isolate specificity: not all chitinolytic bacteria performed equally. Some isolates had moderate or low antagonism and weaker plant growth promotion. This is consistent with reports by Shams et al. (2025), in which even among *Streptomyces* strains certain isolates had stronger VOC or filtrate effects than others. It underscores that screening for multiple traits (inhibition, enzyme activity, growth-promotion) is essential when selecting candidates for biocontrol.

Another important consideration is colonization and persistence in the rhizosphere or plant root tissues. Effective biocontrol requires that the beneficial bacteria survive, proliferate, and maintain activity in the soil environment. The study of KW16 showed strong colonization ability, even translocation into roots and shoots, especially under challenge by *R. solani* (Nowak et al., 2025). Although our greenhouse trial did not measure bacterial population dynamics or colonization explicitly, the strong effects seen imply that ST27d and ST17c are likely able to persist and interact with the plant in vivo. Future studies should examine colonization, survival, and expression of biocontrol genes in situ.

Compatibility with environmental factors and implementation scale is another critical domain. Biocontrol agents must tolerate variable soil pH, moisture, temperature, and native microbial competition. In some recent work, *Bacillus subtilis* Y1336 demonstrated good suppression of *R. solani* under commercial settings (Farhaoui et al., 2024), and *Bacillus velezensis* GH1-13 was effective against multiple *R. solani* anastomosis groups and showed compatibility with fungicides (Lee et al., 2023). These kinds of robustness are necessary if isolates like ST27d and ST17c are to be used in larger scale or field applications.

The implications of this study are several. First, the isolates ST27d and ST17c could be strong candidates for developing biocontrol formulations for soybean stem rot, offering an environmentally friendly alternative to chemical fungicides. This is particularly relevant for sustainable agriculture systems with concerns about fungicide resistance, soil health, and environmental pollution (Enhancing Biotic Stress Tolerance…, 2023; Microbial Bio-Control Agents…, 2024). Second, the dual benefits of disease suppression and growth promotion suggest that application of such bacteria may reduce the need for fertilizers or other growth enhancers if they improve nutrient uptake or plant vigor naturally. Third, given their strong performance in greenhouse settings, these isolates warrant field trials to confirm efficacy under diverse environmental conditions, and to test formulation types, dose, and methods of application.

However, several limitations should be acknowledged. The study was conducted in greenhouse (in planta) settings rather than open field, which may not fully replicate varied environmental stresses and soil biota interactions. Also, while disease incidence, plant height, and leaf number were measured, data on root biomass, physiological parameters (photosynthesis rate, chlorophyll content, stress markers), and molecular plant defense responses were not included. Such parameters could give deeper insight into mechanisms, including confirmation of ISR, or quantification of chitinase gene induction in the plant. Another limitation is that inoculum and bacterial suspension dosages were fixed; exploring dose-response curves could optimize treatments.

In light of these findings, future research should include several aspects. First, *field trials under realistic agronomic conditions* are necessary to test ST27d and

ST17c performance across soil types, moisture regimes, and crop management practices. Second, *microbial population dynamics, colonization, and survival* under field soil and rhizosphere conditions should be studied, perhaps using molecular markers or reporter genes. Third, studies should investigate *mechanisms at molecular level*, including plant gene expression (chitinases, pathogenesis-related proteins, phytohormone signalling), bacterial gene expression (for chitinase, VOCs, siderophores), and interplay with the indigenous microbiome. Fourth, examining efficacy in combination with reduced doses of fungicides or other IPM components may help integrate biocontrol into practical crop protection strategies; recent success with combined biocontrol-fungicide in soybean root rot demonstrates that blending treatments can reduce chemical inputs while maintaining disease control (Enhancing Biotic Stress Tolerance…, 2023). Finally, formulation optimization (carrier materials, shelf life, inoculation timing) will be crucial if these isolates are to be applied by farmers.

Conclusion

This study demonstrated that several chitinolytic bacterial isolates exhibited strong antagonistic activity against *Rhizoctonia solani*, the causal agent of soybean stem rot. Among the isolates evaluated, ST27d and ST17c were the most effective, achieving complete suppression of disease incidence under greenhouse conditions and significantly enhancing plant growth in terms of height and leaf number. These findings highlight the dual role of selected chitinolytic bacteria as both biocontrol agents and plant growth promoters. Their efficacy suggests potential for development into eco-friendly bioformulations that can reduce reliance on chemical fungicides while improving soybean productivity. Future research should focus on field evaluations, mechanistic insights, and formulation strategies to translate these promising isolates into practical tools for sustainable soybean cultivation.

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Authors' Contribution: Priyatin Ulfa did conceptualization and development of the manuscript, and analytical data evaluation, prepared the associated figures and graphs, drafted and critically revised the manuscript, and approved the final version.

Generative AI Statements: The authors declare that this manuscript has been written without the use of generative artificial intelligence tools.

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