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In Vitro Antagonistic Activity of PGPR Against Plant Pathogen

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KEYWORDS

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ABSTRACT:

Wheat (Triticum aestivum L.) stands as a significant global cereal crop, providing a vital source of calories and protein for a substantial segment of the population. Nonetheless, its productivity faces growing limitations due to fungal pathogens, especially Alternaria tenuissima, responsible for leaf spot and blight. These infections result in premature leaf ageing, diminished photosynthetic efficiency, and notable reductions in both grain yield and quality. Furthermore, A. tenuissima generates mycotoxins, which present health hazards to both humans and animals via the food chain. Plant Growth-Promoting Rhizobacteria (PGPR) provide a viable and sustainable option. These advantageous soil microorganisms promote plant development and inhibit pathogens through processes including the synthesis of antifungal substances, lytic enzymes, siderophores, and the activation of systemic resistance.

The present study aims to isolate indigenous PGPR from the rhizosphere of wheat crops affected by A. tenuissima, examine their antagonistic properties in vitro, and assess their viability as biocontrol agents. The results could play a significant role in advancing sustainable methods for controlling A. tenuissima, enhancing crop resilience and productivity, and minimising dependence on chemical fungicides.

Introduction

Wheat (*Triticum aestivum L.*) is a globally important cereal crop, providing a major source of calories and protein for a substantial portion of the world's population (Sharma and Sharma 2025). Nonetheless, its production is considerably impeded by numerous biotic stressors, especially plant pathogenic bacteria such as Fusarium spp., Rhizoctonia solani, and Pythium spp., which induce illnesses including root rot, seedling blight, and damping-off (Gupta et al. 2017). Wheat farming is increasingly jeopardised by several fungal infections, particularly the noteworthy species *Alternaria tenuissima* (Maulenbay and Rsaliyev 2024). This pathogen is linked to leaf spot and blight symptoms, resulting in early leaf senescence, diminished photosynthetic efficiency, and consequently reduced grain yield and quality. *A. tenuissima* generates mycotoxins, which provide health hazards to humans and animals via the food chain (Barak and Schroeder 2012).

The conventional approach to managing fungal infections in wheat predominantly depends on chemical fungicides. Chemical pesticides, while efficacious, have elicited concerns regarding their environmental persistence, the emergence of resistance in diseases, and detrimental impacts on non-target creatures (Akhter et al. 2024). Nonetheless, the excessive and extended application of these compounds has elicited concerns over environmental contamination, the development of disease resistance, and adverse effects on beneficial soil microbes (Wang et al. 2022). Plant Growth-Promoting Rhizobacteria (PGPR) offer a promising biological alternative. PGPR are beneficial soil bacteria that inhabit the rhizosphere and contribute to plant health by promoting growth, enhancing nutrient availability, and most importantly, suppressing plant pathogens (Wang et al. 2021). Their antagonistic activity against phytopathogenic fungi is mediated by a range of mechanisms, including the production of antifungal metabolites (e.g., antibiotics, hydrogen cyanide), lytic enzymes (e.g., chitinases, glucanases), siderophores that

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sequester iron, and the induction of systemic resistance in host plants (Jayaprakashvel et al. 2019; Rodrigo et al. 2021; Yang et al. 2019). In this scenario, there is an urgent necessity for sustainable and environmentally friendly strategies for disease control.

The antagonistic capabilities of PGPR provide them viable options for biological control within integrated pest management (IPM) techniques. Consequently, the local isolation and characterisation of PGPR strains from the rhizospheres of infected crops offer a focused method to identify efficient biocontrol agents tailored to agro-ecological zones (Xu et al. 2025). Recent studies have shown that certain PGPR strains, particularly those belonging to genera such as *Pseudomonas*, (Shen et al. 2013) *Bacillus*, (Malik et al. 2022) and *Azospirillum*, (Cassán et al. 2014) exhibit strong inhibitory effects against *Alternaria* (Attia et al. 2020) species. However, the effectiveness of these strains can vary with environmental conditions and pathogen diversity, making it essential to isolate and screen locally adapted PGPR strains.

This study aims to identify indigenous PGPR from the rhizosphere of wheat crops impacted by *Alternaria tenuissima*, evaluate their antagonistic activity in vitro, and assess their potential as biocontrol agents. The results may facilitate the formulation of sustainable, environmentally friendly approaches for managing *A. tenuissima* infections, thereby enhancing crop resilience, increasing production, and diminishing dependence on chemical fungicides.

Materials and Methods

Plant fungal pathogen and antagonistic strains

The phytopathogenic fungal strain (*Alternaria tenussima* MTCC 2802), were obtained from the microbial type of culture collection and gene bank (MTCC) CSIR-Institute of Microbial Technology, Chandigarh. prior to being stored in the refrigerator at 4 °C until they were needed again, the fungal strains were subculture on potato dextrose agar (PDA) slant and incubator for 5 days at 25 °C + 0.2 °C. A single spore colony was cultured on a PDA plate and incubated for up to seven days at 25 ± 1 °C to assess its morphological characteristics. Observations were made regarding the colony's shape, colour, growth pattern, and margins.

Evaluation of fungal antagonistic activity (Dual culture technique)

The antagonistic activity of *Alternaria tenussima* against fungal pathogen which causes black point disease of wheat was evaluated by using the dual culture technique. Fungal antagonistic strains were injected onto PDA plates using mycelial discs (6 mm in diameter), which were positioned 1.5 cm from the edge of newly made PDA plates. Similarly, 1.5 cm from the edge of the prepared PDA plates, 6mm mycelial disc of fungal phytopathogens were positioned in the opposite direction of the antagonistic fungal strain for five days, the treated plates were incubated at 25 °C + 0.2 °C while the control plate were incubated with the fungal pathogen *Alternaria tenuissima*. The following formula was then used to determine the inhibition percentages and assess the radial growth of the phytopathogen in the control and treated plates.

$$\text{Percentages of inhibition} = \frac{(A - B)\ X\ 100}{A}$$

A-Colony diameter of the phytopathogen in control plate

B-Colony diameter in the dual culture plate antagonistic activity of PGPR micro/isolate against plant fungal pathogen *alternaria*.

Results

The fungal strain *A. tenuissima* MTCC 2802 exhibited characteristic colony growth and morphology under the described culture conditions. After incubation on potato dextrose agar (PDA) plates at 25 ± 1 °C for seven days, colonies reached approximately 5 cm in diameter and developed a distinctive loose, cottony texture. The colony surface displayed a brown coloration, sometimes appearing whitish to ash-grey in the early stages, and gradually darkened as pigmentation increased with time.

Morphologically, colonies often showed a concentric ring pattern, reflecting regular sporulation zones across the agar surface. The margins of the colonies were irregular and somewhat feathery, with diffuse borders extending outward into the medium. Within the colony, sporulation was observed, notably as golden-brown conidia developing in chains at areas most exposed to light. These observations regarding colony shape, color, growth pattern, and margins confirm the identity and

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cultural characteristics of *Alternaria tenuissima* which is all plate observation results show in figure number 1.

To assess urease activity, cultures were grown in broth medium containing urea. The presence of urease was indicated by a change in broth color from yellow to deep pink (cerise/red). Cultures of *A. tenuissima* MTCC 2802 developed a pronounced deep pink coloration, demonstrating a positive urease reaction through the production of urease enzyme and subsequent urea degradation. In contrast, negative control cultures exhibited no colour change and remained yellow, indicating a lack of urease activity. These results confirm that the *A. tenuissima* MTCC 2802 strain is capable of enzymatically degrading urea, consistent with positive urease activity.

Phosphate solubilization was assessed using solid medium supplemented with tricalcium phosphate. The development of clear halos around the colonies indicated active solubilization of insoluble phosphate. The solubilization index, determined by the ratio of halo diameter to colony diameter after incubation, confirmed the strain's ability to release soluble phosphorus, as the colonies formed distinct transparent zones on the medium (P1- 14mm, P2- 24 mm, P3- 12 mm, P4- 40 mm) as show in figure 2. These observations demonstrate the functional trait of *A. tenuissima* MTCC 2802 as both a urease producer and a phosphate solubilizer.

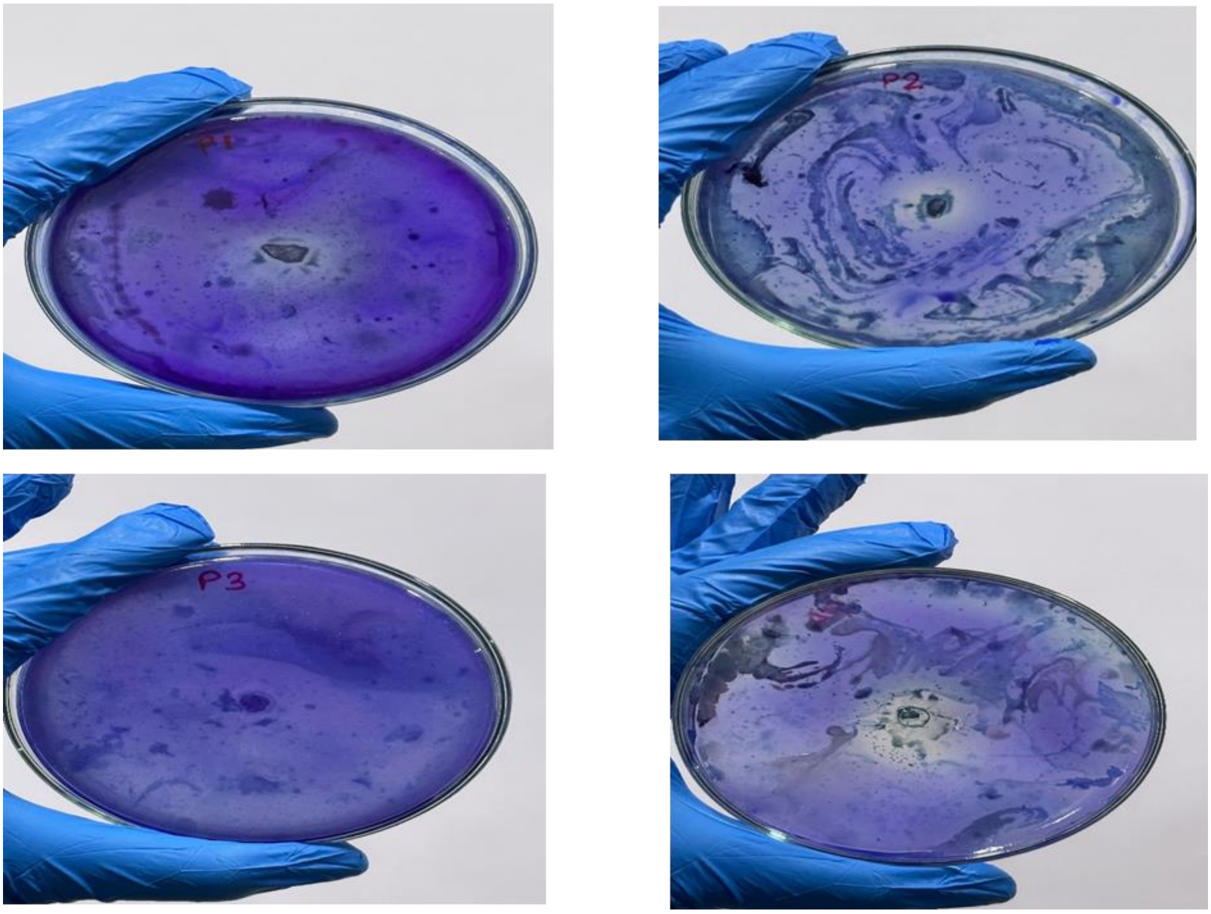

Fig:1 Phosphate solubilizing stages

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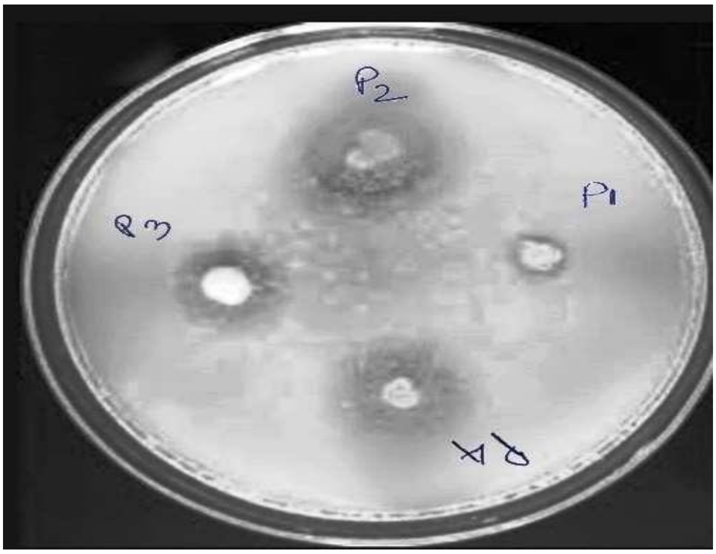
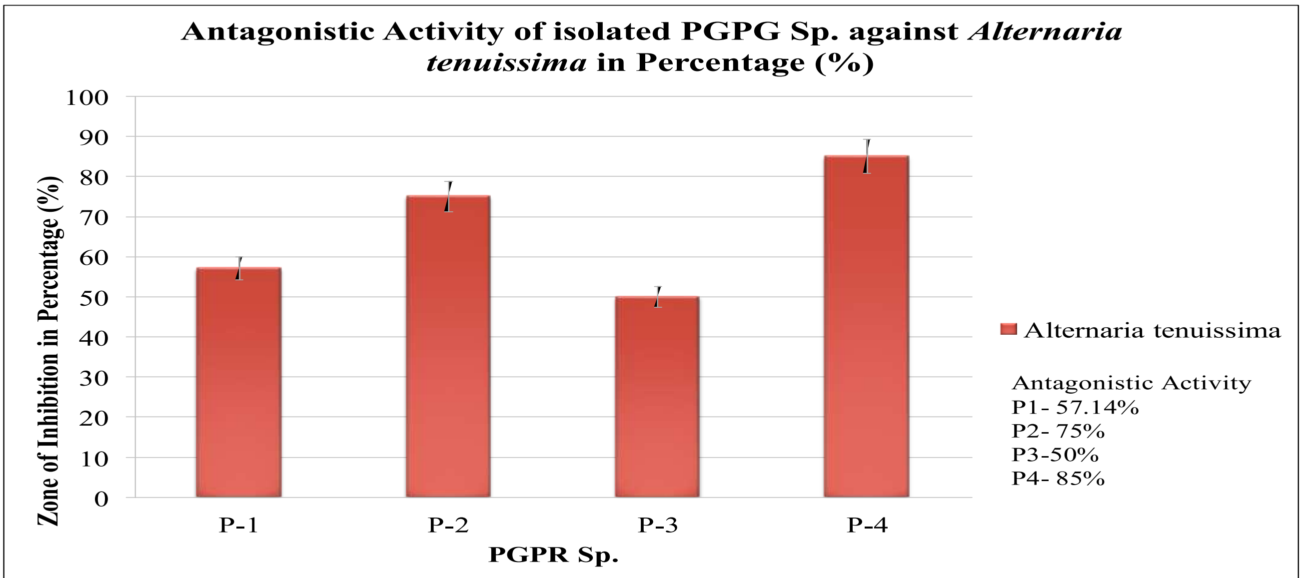


Fig: 2 Showing the image of the dual assay plate method Antagonistic Activity



Graph: 1 Antagonistic activity of four different *Bacillus* strains against *Alternaria tenuissima* under laboratory condition

Discussion

Wheat is a staple crop integral to global food security, yet its productivity is severely threatened by fungal pathogens such as *Alternaria tenuissima*. This fungus is notorious for causing leaf spot and blight, leading to premature leaf senescence and reduced photosynthesis, ultimately diminishing grain yield and quality. Furthermore, the mycotoxins produced by *A. tenuissima* represent a significant risk to human and animal health via the food chain, underscoring the importance of effective disease management (Salimova et al. 2021).

Chemical fungicides are commonly used to control such fungal infections; however, their overuse raises serious environmental and resistance concerns (Islam et al. 2024). There is growing interest in sustainable alternatives like plant growth-promoting rhizobacteria (PGPR), which enhance plant health not only by promoting growth but also by antagonizing pathogens through multiple mechanisms including enzyme production and induced systemic resistance (Meena et al. 2020).

Our study confirms the typical morphological characteristics of *A. tenuissima* MTCC 2802, supporting its identification. Importantly, this strain demonstrated significant urease activity, as evidenced by a color shift in urea broth, indicating its enzymatic capacity to degrade urea. This activity may contribute to the pathogen's environmental adaptability and virulence. Additionally, phosphate solubilization by this strain was confirmed through halo formation on tricalcium phosphate media, indicating its ability to mobilize essential nutrients in the soil.

These dual functional traits of *A. tenuissima* complicate the rhizosphere dynamics, highlighting the necessity for biocontrol strategies that consider such microbial versatility (Giannelli et al. 2024). The antagonistic potential of locally isolated PGPR strains against *A. tenuissima* offers a promising avenue for developing eco-friendly management practices (BiBi et al. 2023). Future research should focus on validating these biocontrol agents under field conditions, integrating them into holistic wheat disease management to reduce reliance on harmful chemicals and improve crop resilience.

Overall, this study underscores the need for integrated, sustainable approaches to safeguard wheat production and mitigate health risks associated with fungal contamination.

Conclusion

Alternaria tenuissima is a significant fungal pathogen threatening wheat production by causing leaf spot and blight, which reduce photosynthesis and yield quality. Our study confirmed the typical morphological features of *A. tenuissima* and demonstrated its positive urease activity and phosphate solubilization ability, traits that may enhance its survival and pathogenicity in the wheat rhizosphere. Given the environmental and health risks associated with chemical fungicides, exploring sustainable alternatives like plant growth-promoting rhizobacteria for biocontrol is crucial. The findings provide a foundation for developing eco-friendly management strategies to mitigate *A. tenuissima*-induced losses and improve wheat crop resilience.

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