

EXPLORING PGPR PROPERTIES AND BIOCONTROL POTENTIAL OF BACTERIAL ISOLATES FOR WHEAT GERMINATION

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ABSTRACT

Wheat (*Triticum aestivum*) is a staple food crop globally, and various biotic and abiotic stresses significantly impact its productivity. The current study aimed to determine the ability to survive bio-control bacteria with antifungal action against 10 phytopathogens, as well as analyze their growth-promoting function in wheat seed growing conditions. Both qualitative and quantitative assays were performed to assess the fungicidal activity of isolated bacteria using the dual culture technique.

Plant growth-promoting features such as Indole 3-Acetic Acid (IAA), phosphate solubilization, and Hydrogen Cyanide (HCN) were evaluated for four biocontrol bacterial isolates, based on 16S rRNA gene sequence analysis. Most of the rhizobacteria that inhibited the growth of the pathogen were identified as *Pseudomonas*, *Bacillus*, and *Actinomyces* out of 20 Isolates, and were chosen for seed germination studies.

KEYWORDS: Biological control, Plant growth-promoting rhizobacteria, Antifungal activity, Wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) remains a cornerstone of global food security, with its cultivation history tracing back to 10,000 BCE in the Fertile Crescent [9]. Since from the domestication of Einkorn and Emmer grasses to its spread across ancient civilizations, wheat has transitioned from a wild-collected grain to a vital global staple [5,26]. While global production generally increased between 1990 and 2024, recent data from the FAO and USDA show a production decrease of approximately 4 million metric tons in the 2023/2024 cycle [24]. India stands as the world's second-largest producer, contributing 13.53% of global output over the last two decades. Within India, Uttar Pradesh leads production (31.35%), followed by Madhya Pradesh (18.18%) and Punjab (16.33%), with high yields supported by fertile alluvial soils and advanced agricultural infrastructure [2,1].

Despite its importance, wheat yields are severely compromised by biotic and abiotic stressors. Fungal diseases alone account for yield losses ranging from 15% to 20%, occasionally exceeding 50% in severe outbreaks [21]. Annually, pests and diseases consume nearly 21.47% of the global wheat crop [23]. Historical management relied on chemical interventions like the Bordeaux mixture; however, concerns regarding phytotoxicity, environmental contamination, and the development of fungicide-resistant strains have limited its modern application [6]. In contrast, India has a successful history of utilizing biocontrol agents to manage agricultural pests and diseases, as summarized in Table 1.

Historical Success of Biological Control Agents in India

Biological control has a long and well-documented history in India and has emerged as an important component of environmentally sustainable pest and weed management. One of the earliest successful examples involved the biological suppression of prickly pear (*Opuntia* spp.) using the cochineal insect *Dactylopius ceylonicus*. Subsequently, classical biological control programmes expanded to several invasive weeds and agricultural pests. In particular, *Dactylopius* spp. provided effective suppression of *Opuntia*, while *Zygogramma bicolorata* became an important biological control agent against *Parthenium hysterophorus*. These experiences established the feasibility of introducing, establishing and utilizing host-specific natural enemies for long-term pest and weed suppression in India [16].

A particularly successful example of weed biological control is the management of water hyacinth (*Eichhornia crassipes*) using the phytophagous weevils *Neochetina eichhorniae* and *N. bruchi*. At a heavily infested 20-ha tank in Bengaluru, *N. eichhorniae* resulted in more than 95% clearance of the weed within 32 months. The successful establishment of *Neochetina* spp. in several water bodies subsequently demonstrated the potential of classical biological control for managing invasive aquatic weeds on a large scale [16,13].

During outbreaks of sugarcane *Pyrilla* (*Pyrilla perpusilla*), parasitoids and other natural enemies, particularly *Tetrastichus pyrrillae* and *Epiricania melanoleuca*, were utilized for pest suppression. Similarly, during the occurrence of sugarcane woolly aphid (*Ceratovacuna lanigera*) in Maharashtra and Karnataka, naturally occurring predators such as *Dipha aphidivora* and *Micromus* spp. were recorded and releases of these predators were undertaken in affected areas. [20]

Microbial biological control has provided another important approach in Indian agriculture. *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) has been extensively evaluated against the chickpea pod borer, *H. armigera*, a major pest of chickpea and several other crops. Field experiments in southern India demonstrated that HearNPV treatments could effectively reduce larval populations and pod damage while increasing chickpea yield. Comparative studies also showed that NPV treatment could provide effective suppression of *H. armigera* under suitable field conditions, supporting its incorporation into integrated pest-management programmes [7]

Overall, these historical examples demonstrate the progressive development of biological control in India from classical introductions of parasitoids, predators and herbivorous insects to the utilization of microbial agents such as baculoviruses. The successful establishment of agents such as *Neochetina* spp., *Zygogramma bicolorata*, *Epiricania melanoleuca* and HearNPV illustrates the potential of biological control to reduce pest and weed populations while reducing dependence on conventional chemical control. However, the effectiveness of individual biological control agents can vary with environmental conditions, pest or weed biology and establishment success; therefore, their integration with other components of integrated pest management remains important [19].

There is an urgent need to investigate sustainable, eco-friendly alternatives for disease management in wheat. Plant Growth-Promoting Rhizobacteria (PGPR) represent a diverse group of beneficial soil bacteria that colonize roots and promote growth through phytohormone production, nutrient solubilization, and the suppression of phytopathogens [10,8,11]. By harnessing PGPR as biocontrol agents, chemical dependency can be minimized, thereby preserving agricultural ecosystems and reducing pollution [3,15].

The present study was undertaken to isolate and characterize PGPR from the rhizosphere of healthy wheat plants. Furthermore, the investigation evaluates their potential to enhance seed germination and suppress prevalent fungal pathogens, contributing to the development of robust biopesticides for sustainable wheat production.

MATERIALS AND METHODS:

1. Sampling And Isolation Of Pgpr

The present Study was conducted between February 2022 and March 2022. Rhizosphere soil samples were collected from *Cicer arietinum* (chickpea) fields in the Chhatrapati Sambhajnagar region. Plants were carefully uprooted, and the soil adhering to their roots was collected as rhizosphere soil. The samples were transferred to sterile zip-lock bags and brought to the laboratory for further processing.

For isolation of rhizobacteria, 1 g of rhizosphere soil was suspended in 9 mL of distilled water. An aliquot of approximately 0.1 mL of the suspension was inoculated onto nutrient agar medium and incubated for the development of bacterial colonies. The bacterial isolates obtained were subsequently purified and maintained for further screening.

Four rhizobacterial isolates designated as PA1, PA2, PA3, and PA4 were subsequently evaluated for their antagonistic activity and plant-growth-associated effects.

2. Bacterial Identification

The isolate was identified based on morphological, culture, biochemical and molecular characteristics using 16S rRNA nucleotide sequencing at National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. The 16S rRNA nucleotide sequence obtained was compared with database sequences using the BLASTn Programme available at the NCBI server (<http://www.ncbi.nlm.nih.gov/BLAST>). The identification was based on the sequence similarity and the closest matching bacterial species available in the database. The isolate was assigned to the species showing the highest sequence similarity and the best BLAST match. The nucleotide sequence of isolates was submitted to the GenBank database to obtain accession number.

3. Preparation of Fungal Phytopathogens

The fungal phytopathogens used for screening were maintained on potato dextrose agar (PDA) medium. The PDA medium was prepared using potato, glucose, agar and sterile distilled water. The fungal cultures were maintained under suitable cultural conditions before their use in the antagonistic assay. The culture procedure was based on the method reported [14], with appropriate modifications.

Ten fungal phytopathogens were evaluated in the antagonistic assay:

- F1: *Curvularia lunata*
- F2: *Chaetomium globosum*
- F3: *Penicillium* sp.
- F4: *Phomopsis* sp.
- F5: *Alternaria alternata*
- F6: *Aspergillus flavus*
- F7: *Fusarium oxysporum*
- F8: *Macrophomina phaseolina*
- F9: *Sclerotium rolfsii*
- F10: *Colletotrichum capsici*

3. Screening of Rhizobacteria for Antagonistic Activity

The antagonistic activity of the rhizobacterial isolates against fungal phytopathogens was evaluated by the dual-culture technique described [22]. Bacterial and fungal cultures were inoculated on the same nutrient agar plate.

The bacterial culture was streaked approximately 4 cm in length on one side of the plate, while the fungal culture was inoculated on the opposite side, maintaining an approximately 15-mm distance from the periphery of the plate.

The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ for 5–7 days. Antagonistic activity was assessed based on the suppression of fungal growth in the presence of the bacterial isolate. The presence of a clear inhibition of fungal growth was considered evidence of antagonistic activity.

The percentage inhibition of fungal growth was calculated using the following formula:

$$\text{Percentage inhibition} = [1 - (\text{fungal growth in treatment} / \text{fungal growth in control})] \times 100$$

The observations obtained for the different bacterial–fungal combinations were recorded and compared with the corresponding fungal control.

4. Wheat Seed Germination Assay

The effect of the selected rhizobacterial isolates on wheat seed germination was evaluated using *Triticum aestivum* seeds. The seeds were surface sterilized by treatment with 70% ethanol for 2 min, followed by treatment with 0.2% HgCl_2 for 3 min with occasional stirring. The sterilized seeds were subsequently used for the germination experiment. Seeds treated with sterile distilled water served as the control.

Four experimental treatment conditions were considered:

1. Seeds treated with fungal culture only.
2. Seeds treated with rhizobacterial culture only.
3. Fungus-treated seeds subsequently treated with rhizobacterial culture.
4. Seeds treated only with sterile distilled water, serving as the control.

The seeds were placed equidistantly on germination paper plates and incubated at room temperature under dark conditions. The experiment was performed in triplicate.

5. Germination

Wheat seeds 450 (*Triticum aestivum*) were surface sterilized by immersion in 70% ethanol for 2 minutes and further in 0.2% HgCl_2 for 3 minutes with occasional stirring. Also, the seeds treated with sterile distilled water served as a control. Then the four sets were prepared: 1) Seeds treated only with the fungus. 2) Seeds treated only with bacteria. 3) Seeds coated first with fungus and treated with bacteria. 4) Seeds were neither treated with fungus nor bacteria, only treated with sterile distilled water, as a control. Seeds were placed equidistantly on germination paper plates and incubated at room temperature in dark conditions. The experiment was done in triplicate. Then the number of germinated seeds was counted after 8 days, and the percent germination was calculated using the formula: $\text{GP} = \frac{N_i}{N} \times 100$

Where:

N_i is the number of seeds germinated.

N is the total number of seeds.

6. Calculation of Seed Germination Percentage

Seed germination percentage was calculated according to the following equation:

$$\text{Germination percentage (GP)} = (N_i / N) \times 100$$

where:

N_i = number of germinated seeds

N = total number of seeds used in the treatment.

Statistical analysis:

Statistical analysis performed using Microsoft Excel 16[Microsoft® Excel® 2021 MSO (Version 2412 Build 16.0.18324.20092) 64-bit]. The experiments were performed independently in triplicate. Mean values of the data for the inhibition of seed germination of each fungal pathogen were compared with the control using the standard deviation

Bacterial isolates were identified previously, but their taxonomic identities are not reported in this manuscript.

Result:

1. Sampling and Isolation Identification of Bacterial Isolates

The bacterial isolates showing promising plant growth-promoting and biocontrol characteristics were selected for molecular identification. The isolates were identified based on their 16S rRNA gene sequences and comparison with the available sequences in the NCBI database. The obtained sequences were compared with reference sequences using the BLAST search, and the closest matching bacterial species were determined on the basis of sequence similarity. As shown in table 1.

Table 1: Identified Isolates

Sr. no.	Isolates code	PGPR Isolates	GenBank Accession No.
1	PA1	<i>Pseudomonas stutzeri</i>	OP649739
2	PA2	<i>Bacillus licheniformis</i>	OM977118

3	PA3	<i>Bacillus amyloliquefaciens</i>	OQ780768
4	PA4	<i>Streptomyces mutabilis</i>	MW575257

2. Plant Growth Promoting Rhizobacteria

The isolated rhizobacterial strains were screened for different plant growth-promoting (PGPR) attributes. The selected isolates exhibited multiple beneficial traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, zinc solubilization, ammonia production, and hydrogen cyanide (HCN) production. The results indicated that the isolates possessed multiple plant growth-promoting and potentially biocontrol-related characteristics. However, the detailed quantitative data for these individual traits are not presented in the present manuscript.

3. In Vitro Antagonistic Activity of Rhizobacterial Isolates

The four rhizobacterial isolates, PA1, PA2, PA3, and PA4, were screened for their antagonistic activity against ten fungal phytopathogens using the dual-culture method. The assay was used to determine whether the bacterial isolates could suppress the growth of the tested fungal pathogens.

The results demonstrated considerable variation in antagonistic activity among the bacterial isolates. PA1 and PA3 showed the broadest antagonistic activity against the tested fungal pathogens, whereas PA2 and PA4 exhibited activity against a comparatively smaller number of fungal isolates. The recorded observations are summarized in Table 2

Table 2. In vitro antagonistic activity of rhizobacterial isolates against fungal phytopathogens (5Days)

Rhizobacteria Fungus	PA1	PA2	PA3	PA4
F1	+	+	+	-
F2	-	-	+	-
F3	+	+	+	-
F4	+	+	+	-
F5	+	+	+	-
F6	+	+	-	-
F7	+	+	+	+
F8	+	+	+	-
F9	+	+	+	-
F10	+	+	+	-

(Note: + fungal growth inhibition, - No fungal growth inhibition)

The quantitative observations further indicated differences in the degree of fungal growth suppression among the bacterial isolates. For example, inhibition values were recorded for *Curvularia lunata*, *Chaetomium globosum*, *Penicillium* sp., *Phomopsis* sp., *Alternaria alternata*, *Aspergillus flavus*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Colletotrichum capsici*. The reported values demonstrate that the magnitude of inhibition varied according to both the bacterial isolate and the fungal pathogen.

Overall, PA1 and PA3 demonstrated comparatively broader antagonistic activity than PA2 and PA4. This indicates that these isolates possessed greater in vitro biocontrol potential against the tested fungal phytopathogens.

2. Effect of Rhizobacteria on Wheat Seed Germination

The effect of rhizobacterial treatment on wheat seed germination was evaluated following an 8-day incubation period. Seeds treated with rhizobacterial cultures showed germination responses that differed among the bacterial isolates. The control treatment, consisting of seeds treated with sterile distilled water, showed 100% germination according to the observed results.

3. Effect of Fungal Treatment on Wheat Seed Germination

Treating wheat seeds with fungal cultures reduced seed germination compared with the untreated control. The fungal treatment therefore negatively affected wheat seed germination under the experimental conditions used.

This observation provides the experimental context for evaluating the subsequent protective effect of rhizobacterial treatment on fungus-exposed seeds.

4. Effect of Rhizobacteria on Fungus-Treated Wheat Seeds

To evaluate the potential biocontrol effect of the rhizobacterial isolates under pathogen-associated conditions, wheat seeds were first exposed to fungal cultures and subsequently treated with rhizobacterial cultures.

The reported observations showed improved seed germination and reduced disease incidence when fungus-coated seeds were subsequently treated with rhizobacteria. This response was illustrated in Figure 7 and Graph 2.

The results indicate that rhizobacterial treatment may provide a protective effect against the negative influence of fungal infection during the early stages of wheat seed germination. In particular, the antagonistic activity demonstrated in the in vitro assay provides supporting evidence for the observed biocontrol response during the seed germination experiment.

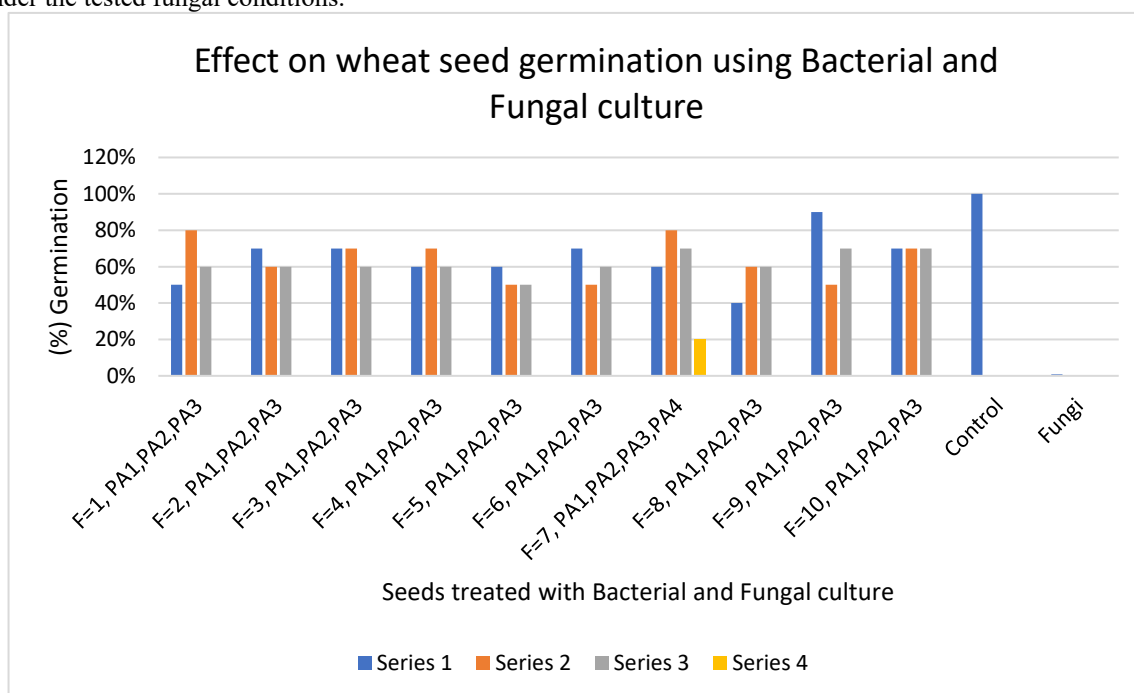
Table 3. Germinated seeds treated with fungi

Sr. no.	Fungal Isolates	Seeds treated with only fungi (Control) out of 10 seeds	Germinated (PGPR+Fungi) seeds			
			PA1	PA2	PA3	PA4
1	F1	1	5	8	6	0
2	F2	3	7	6	6	0
3	F3	1	7	7	6	0
4	F4	0	6	7	6	0
5	F5	0	6	5	5	0
6	F6	2	7	5	6	0
7	F7	1	6	8	7	2
8	F8	0	4	6	6	0
9	F9	0	9	5	7	0
10	F10	1	7	7	7	0

Table 3; Represents the average of 3 independent experiments

Wheat Seed Germination:

The wheat seed germination assay revealed considerable variation among the rhizobacterial isolates under fungal challenge. PA1 exhibited the lowest mean germination response, with 0.90 ± 0.72 germinated seeds, indicating poor performance under the tested conditions. PA2 and PA3 showed the highest mean germination, with 6.40 ± 1.00 germinated seeds for each isolate, whereas PA4 exhibited a slightly lower mean of 6.20 ± 0.48 germinated seeds. These results indicate that PA2 and PA3 were associated with better maintenance of wheat seed germination under the tested fungal conditions.



Graph 2: Effect on wheat seed germination using Bacterial and fungal cultures

7. The Biocontrol Experiment:

The dual-culture assay and wheat seed germination experiment demonstrated that the tested rhizobacterial isolates differed in their ability to suppress fungal growth and influence seed germination. PA1 and PA3 showed

comparatively broader antagonistic activity against the fungal phytopathogens, while rhizobacterial treatment of wheat seeds was associated with improved germination compared with fungus-treated seeds. The combined observations suggest that the selected rhizobacterial isolates possess in vitro antagonistic and potential biocontrol properties, warranting further characterization of their plant-growth-promoting and pathogen-suppressive mechanisms.

Table 3. In vitro antagonistic potential of rhizobacteria against the 10 fungal phytopathogens. (5Days)

Fungi	Fungal Growth On PDA (mm)	Growth in dual culture with bacterial isolate fungal growth inhibition (%)			
		PA1	PA2	PA3	PA4
Curvularia lunata	90	50%	80%	60%	-
Chaetomium globosum	90	75%	65%	65%	-
Penicillium sp.	90	70%	75%	75%	-
Phomopsis sp.	90	65%	75%	65%	-
Alternaria alternata	90	64%	55.5%	63.2%	-
Aspergillus flavus	90	72%	55%	60%	-
Fusarium oxysporum	90	60%	85%	72%	43%
Macrophomina phaseolina	90	40%	65%	66.6%	-
Sclerotium rolfsii	90	88%	52%	70%	-
Colletotrichum capsici	90	72%	72%	75%	-

8. Antagonistic potential of rhizobacteria against 10 fungal phytopathogens



Fig.1 Fig.2 Fig.3 Fig.4 Fig.5 Fig.6 Fig.7 Fig.8 Fig.9 Fig.10

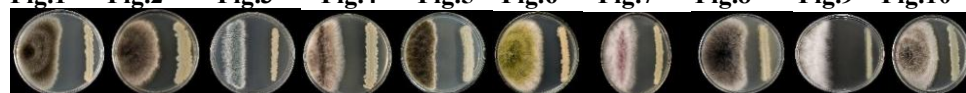


Fig.11 Fig.12 Fig.13 Fig.14 Fig.15 Fig.16 Fig.17 Fig.18 Fig.19 Fig.20



Fig.21 Fig.22 Fig.23 Fig.24 Fig.25 Fig.26 Fig.27 Fig.28 Fig.29 Fig.30

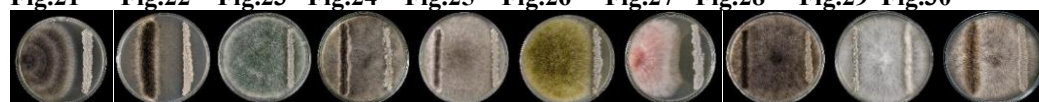


Fig.31 Fig.32 Fig.33 Fig.34 Fig.35 Fig.36 Fig.37 Fig.38 Fig.39 Fig.40

Fig. 1-10; Antagonistic activity of bacterial isolate PA1(*Pseudomonas stutzeri*) against different fungal phytopathogens using the dual-culture assay. Fig. 11-20; PA2(*Bacillus licheniformis*) against different fungi, Fig. 21-30; PA3(*Bacillus amyloliquefaciens*) against different fungi, Fig. 31-40; PA4(*Streptomyces mutabilis*) against different fungi.

8. Wheat Seed Germination using Bacterial and Fungal Culture -

Fig. 41 and Graph 2 demonstrated a significant reduction in disease incidence and a notable improvement in germination rates when seeds pre-coated with fungi were subsequently treated with Rhizobacteria



Fig. 41: The effect of rhizobacteria on germination of fungus-coated wheat seeds and disease incidence reduction in wheat seeds and its biocontrol activity.

CONCLUSION

The ability of rhizobacteria to directly oppose phytopathogens and enhance host immunity through the in vitro antifungal activity of rhizobacterial strains. By producing phytohormones, antagonistic isolates can also directly exhibit important traits that promote plant development. In order to assess the impact of fungal culture on the germination of wheat seeds, the first treatment of seed germination using fungal culture was carried out, and the results clearly showed suppression of wheat seed germination. Rhizobacteria demonstrated a considerable improvement in the germination rate of wheat seeds after the second treatment of seed germination using rhizobacterial culture. The purpose of the third treatment, which involved the germination of wheat seeds using bacterial and fungal cultures, was to ascertain the impact of bacterial culture on the development of fungal-infected wheat seeds. This procedure showed that bacterial culture had a positive impact on wheat seedlings. Significant differences have been seen between fungal-infected seeds and seeds treated with rhizobacteria and phytopathogens, indicating that rhizobacterial culture both concurrently boosts plant development and inhibits the detrimental effects of fungal pathogens.

Therefore, Rhizobacteria can be used to improve wheat seed germination and plant growth promotion. Based on their direct and indirect plant growth-promoting traits and antagonistic potential, the isolates can be valuable input and can be further studied for diverse biotechnological applications besides biofertilization and biocontrol

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