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Biocontrol Approaches in Reducing *Tilletia spp*. Infections in Common Wheat

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ABSTRACT

Wheat common bunt, caused by *Tilletia spp*., remains an important disease of wheat, particularly in organic and low-input production systems where chemical seed treatments are limited. The present study evaluated the biocontrol potential of three indigenous Romanian *Pseudomonas* isolates against wheat common bunt under laboratory and field conditions. Bacterial strains were isolated from agricultural soils in Bucharest and Ilfov County and identified through morphological, biochemical, and 16S rRNA gene analyses as *Pseudomonas fluorescens* (Ps.F), *Pseudomonas putida* (Ps.P), and *Pseudomonas* sp. (Ps.R). The isolates exhibited several traits associated with plant growth promotion and biological control, including hydrolytic enzymes, siderophore synthesis, and phosphate solubilization. Antagonistic activity against *Tilletia* spp. was assessed by evaluating teliospore germination inhibition *in vitro*. *Pseudomonas putida* showed the strongest inhibitory effect, reducing teliospore germination from 51.85% in the untreated control to 7.14%, corresponding to 86.2% inhibition, followed by *Pseudomonas fluorescens* with 59.8% inhibition. Field trials were conducted under organic farming conditions using four common bunt-susceptible wheat cultivars (Armura, FDL Columna, Izvor, and Pitar). Disease suppression varied among cultivars, indicating a significant strain-cultivar interaction. Ps.P provided the highest protection, achieving up to 76.6% efficacy in cultivar FDL Columna, while Ps.F reduced common bunt incidence by more than 50% in Armura. *Pseudomonas* sp. Ps.R exhibited comparatively lower and cultivar-dependent effectiveness. These findings demonstrate that native *Pseudomonas* strains, particularly of *P. putida* and *P. fluorescens*, represent promising candidates for the development of environmentally sustainable biological seed treatments for wheat common bunt management in organic production systems.

Keywords: biocontrol, bunt disease, organic farming, *Pseudomonas*, seed treatment, *Tilletia*, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) represents one of the most important cereal crops worldwide, serving as a primary source of carbohydrates and protein for a significant proportion of the global population. Maintaining stable wheat production is therefore essential for global food security. However, crop productivity is continuously challenged by fungal diseases that reduce grain yield and quality. Among these diseases, common bunt and dwarf bunt, caused by species of the genus *Tilletia*, remain important constraints in many wheat-growing regions, particularly under organic and low-input agricultural systems where chemical seed treatments are restricted or limited (Jia et al., 2022).

Common bunt is mainly caused by *Tilletia caries* and *T. laevis*, while dwarf bunt is associated with *T. controversa*. These pathogens infect young wheat seedlings shortly after germination and complete their life cycle within the host tissues. At maturity, infected kernels are replaced by sori filled with dark masses of teliospores responsible for the characteristic fish-like odor associated with bunted grain (Gurjar et al., 2023). Besides causing yield losses, bunt infection severely reduces grain quality and marketability, making disease management a priority for producers and seed certification systems (Protic et al., 2011; Jia et al., 2022).

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The disease cycle of bunt fungi is initiated by teliospores contaminating seeds or soil. Under favorable environmental conditions, teliospores germinate to produce promycelia and infective sporidia capable of colonizing emerging wheat seedlings. Because teliospore germination represents a critical stage in disease establishment, the inhibition of this process has become a major target in the development of alternative disease control strategies (McManus et al., 1993; Wen et al., 2026).

Historically, bunt control has relied heavily on chemical seed treatments, which remain highly effective in reducing disease incidence. Nevertheless, increasing environmental concerns, restrictions on pesticide use, and the growing demand for organic agricultural products have stimulated interest in environmentally friendly alternatives. Furthermore, sustainable disease management increasingly requires integrated approaches that combine host resistance, agronomic practices, and biological control methods (Bakumenko et al., 2025; Hanczig et al., 2025).

During the last decades, biological control has emerged as a promising strategy for suppressing soil- and seed-borne plant pathogens. Various microorganisms, including fungi and bacteria, have demonstrated the ability to inhibit pathogen development through mechanisms such as competition for nutrients, antibiosis, siderophore production, hydrolytic enzyme secretion, and induction of plant defense responses (Ali et al., 2014; Shams-Allah et al., 2016; Shen et al., 2024). Recent studies have further highlighted the importance of the plant microbiome and rhizosphere microbial communities in determining plant health and disease resistance, emphasizing the role of beneficial microorganisms in sustainable crop production (Jia et al., 2022; Shen et al., 2024). Evidence supporting the potential of microbial-based crop protection strategies has also been reported by Petcu et al. (2023), who investigated the efficacy of *Trichoderma harzianum* and *Bacillus subtilis* applied as seed and foliar treatments in maize, in combination with agroecological management measures.

Among the bacterial genera investigated for plant disease management, *Pseudomonas* occupies a prominent position due to its remarkable metabolic versatility and ecological adaptability. Species such as *Pseudomonas fluorescens* and *Pseudomonas putida* are widely distributed in agricultural soils and plant rhizospheres, where they contribute to nutrient cycling, plant growth promotion, and pathogen suppression. Their biocontrol activity is frequently associated with the production of siderophores, antibiotics, lytic enzymes, volatile organic compounds, and other secondary metabolites capable of inhibiting fungal development (McManus et al., 1993; Asthana et al., 2026).

Previous investigations have demonstrated the ability of fluorescent pseudomonads to suppress bunt pathogens through direct inhibition of teliospore germination and interference with pathogen establishment. For example, McManus et al. (1993) reported that *Pseudomonas fluorescens* strain 2-79 significantly inhibited germination of *Tilletia laevis* teliospores and reduced common bunt severity under experimental conditions. More recently, Asthana et al. (2026) confirmed the antagonistic activity of *P. fluorescens* against *Tilletia indica*, further supporting the use of pseudomonads as biological control agents against common bunt diseases.

In addition to bacterial antagonists, numerous alternative approaches have been investigated for bunt management, including plant-derived antimicrobial extracts, essential oils, fungal bioagents, induced resistance strategies, and nanomaterials (Castañeda et al., 2001; Ali et al., 2014; Hanczig et al., 2025; Jabran et al., 2025; Smita et al., 2025). Although many of these approaches have shown promising results, their effectiveness may vary depending on environmental conditions, pathogen populations, and wheat genotype. Consequently, the identification of locally adapted biological control agents remains an important objective for sustainable agriculture.

Indigenous microorganisms are particularly attractive candidates because they are naturally adapted to local environmental conditions and may exhibit enhanced survival and

competitiveness when reintroduced into agricultural ecosystems. The screening of native bacterial populations therefore represents an important step toward the development of effective microbial inoculants suitable for regional agricultural practices.

The present study aimed to isolate and characterize indigenous *Pseudomonas* strains from Romanian soils and to evaluate their potential for the biological control of wheat common bunt caused by *Tilletia* spp. The selected isolates were identified through morphological, biochemical, and molecular analyses, and their antagonistic potential was assessed by measuring inhibition of teliospore germination under laboratory conditions. Furthermore, their efficacy as preventive seed treatments were evaluated under field conditions using four common bunt-susceptible wheat cultivars cultivated under organic farming practices. By integrating microbiological characterization with laboratory and field evaluations, this work contributes to the growing body of research focused on developing environmentally sustainable alternatives for the management of common bunt diseases in wheat production systems.

MATERIAL AND METHODS

Bacterial isolation

Three Romanian-native bacterial strains were used in this study (Table 1). The isolation was performed from soil samples collected from Bucharest and Ilfov County. The samples were collected from the active soil layer at the depth of 0-25 cm. Each soil sample was thoroughly homogenized to break apart soil clumps, and 10-gram portions of soil were infused for 1h under orbital shaking in sterile distilled water (SDW). Soil infusions were subjected to serial tenfold dilutions and plated on Plate Count Agar (PCA).

Table 1. Bacterial isolation

Bacterial Strain	Isolation source	
Ps. F	*Bucharest*	44.43885°N, 26.02365°E
Ps. P		44.46063°N, 26.06341°E
Ps. R	*Ilfov County*	44.49890°N, 26.28540°E

Single colonies exhibiting smooth, semi-translucent growth were subsequently purified on King B medium following incubation at 28°C.

Microbiologic analysis of isolated bacteria

Microbial characterization of the bacterial isolates was carried out by examining their microscopic features and metabolic traits through several qualitative biochemical assays performed in triplicate. The biochemical plate assays included catalase activity, starch depolymerization, casein and gelatin hydrolysis, cellulase and chitinase activity, siderophore production, phosphate solubilization, as well as arginine and ornithine decarboxylase activity.

Catalase activity was performed on sterile grass slides by placing one loop of fresh culture onto a drop of 3% hydrogen peroxide. Reactions were considered positive when effervescence was observed (Iwase et al., 2013).

Starch depolymerization assay was performed on nutrient agar supplemented with 0.4% (w/v) soluble starch. After five days of incubation at 28°C, plates were flooded with Lugol's iodine solution. Reactions were considered negative when starch substrate was colored in dark blue around the bacterial growth, indicating the absence of enzymatic degradation. Positive reactions were recorded when a clear halo developed around the colonies, whereas partial hydrolysis was indicated by the formation of a thick, brick-yellowish halo surrounding the bacterial growth.

Proteolytic activity was evaluated on casein and gelatin substrates. Casein hydrolysis was assessed on skim milk agar (SMA), while gelatin hydrolysis was tested on nutrient agar supplemented with 0.4%

(w/v) gelatin. After three days of incubation at 28°C, caseinase activity was considered positive if clear halo developed around the bacterial growth. In contrast, gelatin hydrolysis was visualized after flooding the plates with acidic mercuric chloride solution (15% $HgCl_2$ in 20% HCl), which precipitated the unhydrolyzed protein and revealed the areas where gelatin has been degraded by the bacteria.

Cellulase activity was assessed on 1% (w/v) carboxy-methyl cellulose (CMC) specific medium (Sicuia et al., 2015). After five days of incubation at 28°C, plates were flooded with 0.1% Congo red for 15 min, then rinsed 2 times with 1M sodium chloride solution. Clear halos around the colonies indicated CMC degradation due to bacterial cellulase activity.

Chitinase activity was evaluated on colloidal chitin medium, according to Agrawal and Kotasthane (2009), after five days of incubation at 28°C. Positive reactions were revealed by the bromocresol purple indicator dye, which allowed substrate color shift from yellow (acidic pH) to purple (neutral pH).

Siderophore production was tested on King B medium, to reveal the UV-fluorescent siderophores such as pyoverdine. Chrome azurol S (CAS) medium (Himpsl and Mobley, 2019) was also used to detect siderophores independent of their chemical structure. Positive reactions on CAS medium were recorded when the color of the medium changed from blue to pale orange.

Phosphate solubilization was tested on Pikovskaya medium containing tricalcium phosphate. Solubilization potential of tested bacterial strains was indicated by the formation of clear, translucent or semi-translucid halos around the colonies after seven to ten days of incubation at 28°C.

Arginine Decarboxylase activity was assessed on solid media containing 0.2% (w/v) L-arginine hydrochloride (Boiu-Sicuia et al., 2023). Enzymatic activity was visualized after one day incubation at 28°C, by the color change of the medium from yellow to pink.

Bacterial DNA extraction

Fresh bacterial cultures grown on Luria-Bertani medium were used for genomic DNA extraction. The nucleic acids were isolated using the High Pure PCR Template Preparation Kit (Roche Life Science, Mannheim, Germany), following the manufacturer's protocol.

Bacterial identification

Identification of bacterial strains was performed by amplifying the conserved 16S rRNA gene region. PCR amplification employed the universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) (Ritwik et al., 2025) and 1492R (5′-ACGGCTACCTTGTTACGACTT-3′) (Zhao et al., 2025).

Each reaction mixture contained 100 ng genomic DNA, 1X Taq buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Thermo Scientific, Vilnius, Lithuania), 0.5 μM of each primer (Biolegio B.V., Nijmegen, The Netherlands), and 0.25 U of MangoTaq DNA Polymerase (BioLine, UK), adjusted in MilliQ water up to 50 μL total volume.

Amplification was performed with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1min, primer annealing at 56°C for 1 min, and extension at 72°C for 2 min, followed by a final elongation at 72°C for 10 min. PCR products were verified by agarose gel electrophoresis, purified, and subsequently sequenced using the Sanger dideoxy method (CeMIA, Larissa, Greece). Forward and reverse reads were aligned and curated in BioEdit Sequence Alignment Editor (v7.2.5). Taxonomic assignment was performed using NCBI's NBLAST (Nucleotide BLAST+ 2.17.0), comparing the assembled sequences against the NCBI microbial reference database.

The phylogenetic relationships among the analyzed strains, together with their closest reference taxa, were inferred in MEGA-X v10.1.8 using the Neighbor-Joining algorithm, with evolutionary distances computed according to the Kimura 2-parameter model and node support assessed through 100 bootstrap replicates.

Tilletia spp. population

Wheat kernels naturally infected by common bunt (*Tilletia* spp.) were collected from the experimental fields of the National Agricultural Research and Development Institute (NARDI) Fundulea, Romania. Wheat kernels were surface disinfected with 5% sodium hypochlorite for 25 min, rinsed 5 times with SDW, then dried in sterile air flow. Teliospores were suspended in SDW with Tween 80 and microscopic examined under Nikon Eclipse 2000 LED MV R optical microscope (Nikon Corporation, Tokyo, Japan) to assess the morphology.

In vitro antagonism screening

The streak diffusion assay was used to evaluate the antifungal activity of the three studied bacterial strains against *Tilletia* spp. Tests were performed *in vitro*, on 1.5% (w/v) Water-Agar. Teliospore suspension (10^7 CFU·mL^{-1}) was used to surface inoculate the plates. To stimulate teliospore germination plates were first incubated for 2 h at 4°C, then three days at 15°C. *Tilletia* inoculated plates were then streaked with the studied bacteria and forwardly incubated at 18°C. Positive control plates, with *Tilletia* only, were also considered. Antagonistic activity was evaluated based on the inhibition potential of teliospore germination, observed under microscopic analysis.

Field trials

Field experiments were carried out in small scale test plots at NARDI Fundulea. Four wheat cultivars Armura, FDL Columna, Izvor and Pitar, all bunt-susceptible were used.

The same three bacterial strains previously evaluated under *in vitro* conditions, were used for preventive seed treatment under field conditions. Bacteria were grown in Luria Bertani broth for 2 days, at 28°C, under orbital shaking at 150 rpm. The inoculum was prepared as viable-cell suspensions, standardized at 10^8 CFU·mL^{-1}, supplemented with 2‰ CMC to improve bacterial adhesion to the kernel surface.

The seed treatment was applied by kernel immersion for 30 min in the respective microbial suspensions. While the kernels were still wet, they were subjected to experimental inoculation with *Tilletia* teliospores at a rate of 0.5 g of teliospores per 15 g of kernels. Four experimental variants were established for each wheat cultivar: a *Tilletia* positive control; and three biologic treatments were *Tilletia*-inoculated kernels were preventively treated with one of the studied strain, Ps F., Ps. P and Ps. R, respectively. Each variant was prepared in four biological replicates.

Sowing took place in late October 2025 under organic farming, and plants were harvested in June 2026. Bunt incidence (BI %) was determined at harvest by counting ears exhibiting typical bunt symptoms, namely sori filled with teliospores. Incidence was calculated using the formula:

$$BI\ (\%) = IE / TE \times 100$$

where IE represents the number of infected ears, while TE is the total number of ears.

Besides BI (%) the treatment efficacy (E %) was also calculated considering the BI in the control and in preventive treatment, as follows:

$$E\ (\%) = (BI_C\text{-}BI_T) / BI_C \times 100$$

where BI_C represents the bunt infection in the control variant, while BI_T is the bunt infection in the treatment.

RESULTS AND DISCUSSION

Bacterial identification

Three bacterial strains were selected within this study, named: *Pseudomonas fluorescens* strain Ps.F, *P. putida* strain Ps.P and *Pseudomonas* sp. strain Ps.R, all isolated from soil. Macroscopic analysis revealed smooth, semi-translucid colonies on PCA, while on King B the Ps.F produced green-fluorescent pigmentation under UV light (Figure 1).

Microscopic analysis revealed all three bacteria as Gram negative, with small bacillar shaped cells (Figure 2).

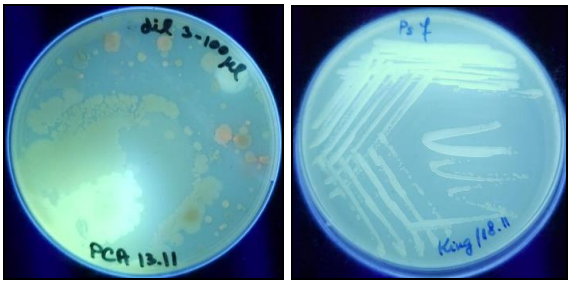


Figure 1. Bacterial isolation (left) and purification (right)

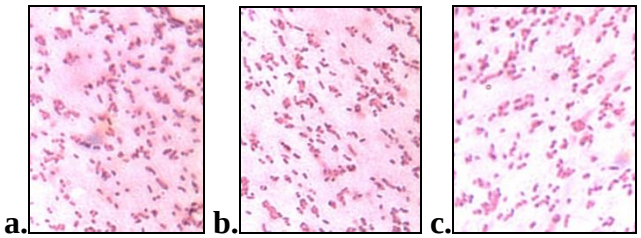


Figure 2. Microscopic view of the isolated Gram-negative bacterial strains:
a. *P. fluorescens* strain Ps.F; b. *P. putida* strain Ps.P; c. *Pseudomonas* sp. strain Ps.R

Genetic identification based on 16S rARN region included these stains in the genus *Pseudomonas*, with a high query cover and identification percentage compared with reference strains from renown international culture collections, with their partial sequence included in the GenBank (Table 2).

Table 2. Molecular identification

Bacterial strain	Scientific name	Query length	Query cover	Identity percent	Accession length	Closest 16S rARN reference strain	GenBank accession number of similar reference sequence
Ps. F	*Pseudomonas fluorescens*	1341 bp	100%	99.85%	1458 bp	*P. fluorescens* strain ATCC 13525 (NBRC 14160, DSM 50090)	NR_114476.1
Ps. P	*Pseudomonas putida*	1394 bp	100%	100%	1462 bp	*P. putida* strain NBRC 3738 (ATCC 8209, DSM 1693)	AB680123.1
Ps. R	*Pseudomonas* sp.	1319 bp	100%	99.66%	1450 bp	*P. japonica* strain NBRC 103040 (DSM 22348)	KT825519.1

Legend: ATCC in the *American Type Culture Collection*; NBRC it the *National Biological Resource Center* of the National Institute of Technology and Evaluation (NITE) Japan; DSM is the *German Collection of Microorganisms.*

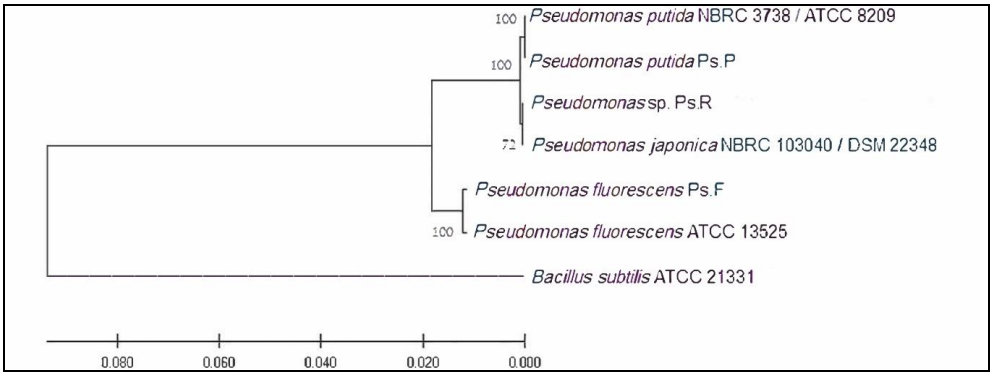


Figure 3. Neighbor-Joining dendrogram showing the phylogenetic relationships among the analyzed *Pseudomonas* strains and their closest reference taxa

The Neighbor-Joining phylogenetic tree revealed three well-supported clades within the analyzed *Pseudomonas* strains (Figure 3). The Ps.P strain was grouped tightly with *P. putida* NBRC 3738 (sin. ATCC 8209, DSM 1693), forming a highly supported clade (bootstrap 100), consistent with its phenotypic assignment. In contrast, strain Ps.R was clustered beside *P. japonica* NBRC 103040 (sin. DSM 22348) with a moderate bootstrap value (72), indicating a close but not fully set relationship. While Ps.F strain was clustered with *Pseudomonas fluorescens* ATCC 13525, also with maximal bootstrap support (100), confirming its affiliation with the *P. fluorescens* lineage. The outgroup *Bacillus subtilis* ATCC 21331 anchored the tree and validated the separation between Gram-negative *Pseudomonas* taxa and the Gram-positive reference.

The branch length analysis indicates that the *Pseudomonas fluorescens* clade exhibited a higher evolutionary divergence (branch length 0.013) compared with the other two clusters, whereas the *P. putida* and *P. japonica* clades showed minimal divergence, with branch lengths very close to the root. This pattern suggests that Ps.F is genetically more distant from its reference strain compared to Ps.P and Ps.R, which cluster tightly with their respective taxa. However, BLAST analysis indicated that Ps.R should remain identified at genus level, as approximately 50% of its closest sequence matches in NCBI were annotated simply as *Pseudomonas* sp., suggesting insufficient resolution for a confident species-level assignment. Most likely, this limited taxonomic resolution is due to the fact that the sequenced 16S rRNA gene fragment was only 1319 bp long, which reduced the discriminatory power of the marker and decreased the likelihood of achieving a reliable species-level affiliation (Table 2).

Biochemical profile of the bacterial strains

The studied strains revealed a wide enzymatic and metabolic activity (Table 3), supporting their biocontrol and biostimulant potential if used as agro-inoculants.

Table 3. Biochemical traits of studied bacterial strains

Enzymatic or metabolic trait	Bacterial strain		
	Ps.F	Ps.P	Ps.R
Catalase activity	+	+	+
Starch depolymerization	–	–	±
Casein hydrolysis	±	–	–
Gelatine hydrolysis	+	–	±
Cellulase activity	±	–	+
Chitinase activity	+	+	+
Siderophore production	+	+	+
Phosphate solubilization	±	±	±
Arginine decarboxylase activity	+	+	–

Legend: + positive, ± weak positive, – negative reaction.

The strains exhibited enzymatic patterns characteristic for soil-associated pseudomonads, with several distinctive traits that support their taxonomic placement and ecological differentiation.

All strains showed strong catalase activity, consistent with the aerobic metabolism typical across *Pseudomonas* species (Abebe et al., 2026). Regarding starch depolymerization, only Ps.R initiated starch hydrolysis (±) after five days of incubation, while Ps.F and Ps.P remained negative. Weak amylase activity or no starch hydrolysis is common among non-specialized pseudomonads (Pagaya et al., 2025).

Regarding proteolytic activity, Ps.F displayed strong hydrolytic potential, with positive reactions for both casein and gelatin hydrolysis (Figure 4). This phenotype is characteristic of *P. fluorescens*, which secretes extracellular proteases involved in nutrient acquisition and antagonistic interactions (Ahmed and Hassanm, 2024). Ps.P was negative for both casein and gelatin

hydrolysis, a pattern reported for *P. putida* strains in the Systematic Bacteriology (Garrity et al., 2005). Ps.R showed weak gelatinase (±) and no caseinase activity, suggesting a reduced proteolytic profile.

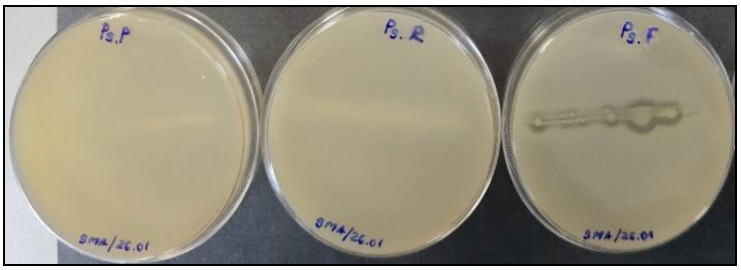


Figure 4. Caseinase activity of pseudomonads on SMA

Cellulase activity was detected in Ps.R, whereas Ps.F showed a lower level (Figure 5). Such findings are in accordance with various studies reporting pseudomonads as producers of cellulases enzymes (Bakare et al., 2005; Sethi et al., 2013; Viswanath et al., 2018).

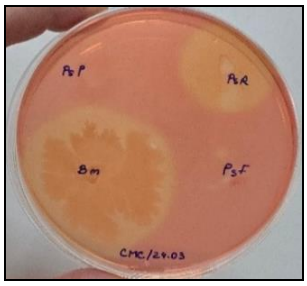


Figure 5. Cellulase activity of pseudomonads on CMC substrate

Chitinase activity was present in all three strains (Figure 6), although Ps.P expressed weaker activity. In *P. fluorescens*, especially in soil and rhizosphere derived bacteria, a high chitinolytic potential is commonly stated (Suganthi et al., 2015; Alhasawi and Appanna, 2017). Studies on *P. putida* reveal that Mg^{2+} and Ca^{2+} can boost chitinase production, whereas Mn^{2+}, Zn^{2+}, and Cu^{2+} are inhibiting enzyme production (Mini et al., 2023).

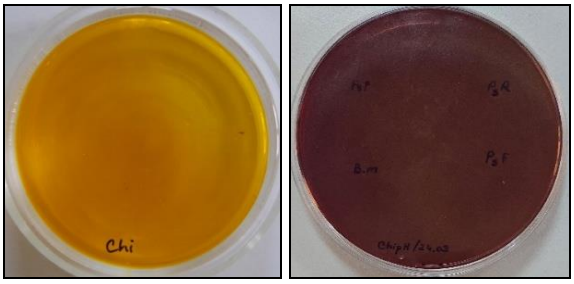
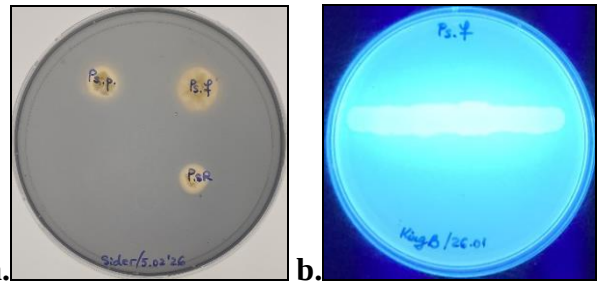


Figure 6. Chitinase activity of pseudomonads on colloidal chitin substrate
- uninoculated control (left) and positive reactions (right) -

All strains produced siderophores (Figure 7a), as indicated by positive reactions on CAS medium, a conserved trait linked to iron acquisition and competitive fitness of pseudomonads in soil environments. Ps.F displayed strong siderophore production, consistent with pyoverdin synthesis (Figure 7b), a hallmark of fluorescent pseudomonads (Garrity et al., 2005).



a. **b.**

Figure 7. Siderophores production on different media
a. CAS agar; b. King B agar

All strains exhibited phosphate solubilization potential, in moderate levels, as typical for many *Pseudomonas* spp., which solubilize inorganic phosphate mainly through organic acid secretion (Rodríguez and Fraga, 1999).

For arginine decarboxylase, Ps.P and Ps.F strains were found positive (Figure 8). This pathway appears to be characteristic in many pseudomonads, as source of polyamines (Garrity et al., 2005).

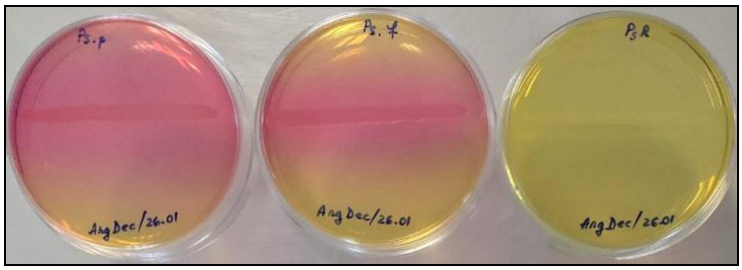


Figure 8. Arginine decarboxylase production by Ps.P and Ps.F strains

Overall, the biochemical profiles of the investigated strains highlighted a combination of hydrolytic enzyme and siderophores production, phosphate-solubilizing capacity, and arginine decarboxylase activity that are closely associated with plant growth promotion and biological control potential. These traits collectively support the potential application of the studied strains as multifunctional bacterial inoculants for sustainable agricultural systems.

Bacterial effect on Tilletia teliosperes germination

The *in vitro* antagonism assay revealed visual differences among the tested bacterial strains regarding their ability to inhibit *Tilletia* teliospore germination (Figure 9).

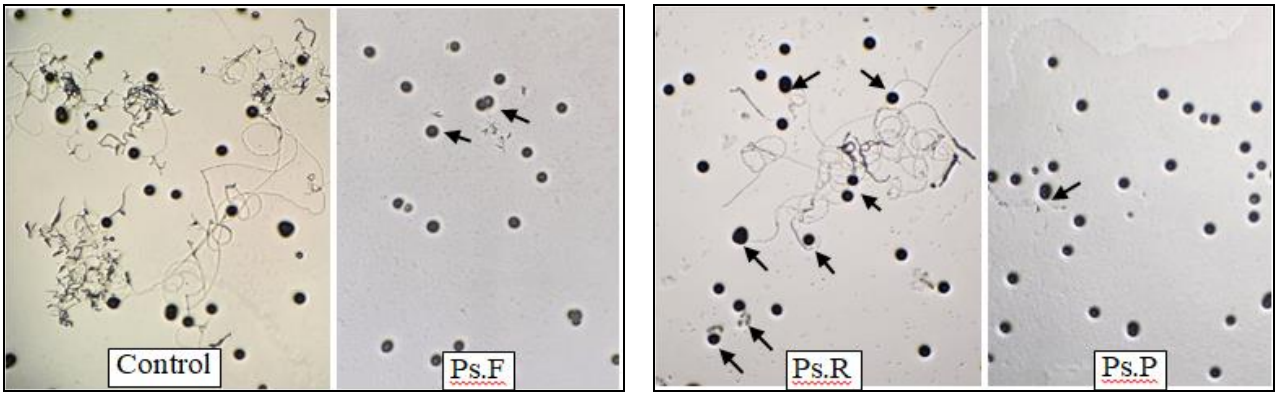


Figure 9. Tilletia teliospores germination after exposure to bacterial treatments

In the untreated control, 51.85% of teliospores germinated. In contrast, the bacterial treatments reduced teliospore germination, except for Ps.R which exhibited only a weak inhibitory effect. The lowest germination rate was observed in the presence of strain Ps.P, where only 7.14% of the teliospores germinated. Strain Ps.F also markedly reduced germination, resulting in 20.83% germination rate, whereas in the presence of strain Ps.R, 45.45% of teliospores germinated.

Teliospores germination represents a critical role in the disease cycle of *Tilletia*, as it leads to the development of promycelium and production of infective sporidia capable of initiating infection in wheat plants (Trione, 1973). Therefore, by inhibiting teliospore germination, the Ps.P and Ps.F strains may reduce the reduce the risk of disease development. However, without a complete inhibition, common bunt infection may still occur under favorable environmental conditions on susceptible cultivars.

Compared with the untreated control, germination inhibition reached 86.2% by Ps.P inoculation and 59.8% with Ps.F, while Ps.R exhibited only limited antagonistic activity, reducing germination by 12.3%.

Similar studies have evaluated the inhibition of *Tilletia* teliospore germination by microbial and plant extracts means (McManus et al., 1993; Castañeda et al., 2001; Wen et al., 2026). Among these, fluorescent pseudomonads have repeatedly demonstrated strong biocontrol potential through the production of antifungal metabolites and other mechanisms that suppress pathogen development (Asthana et al., 2026).

Bacterial efficacy in common bunt prevention

After *in vitro* evaluation of antifungal activity, the bacterial strains were applied as seed treatments on four wheat cultivars to evaluate the biocontrol potential on common bunt infection in field conditions. The results partially confirmed the *in vitro* findings and further suggested that bunt severity was influenced by both the applied treatment and wheat cultivar. The observed differences among cultivars suggest that the biocontrol efficacy of the bacterial treatments may be influenced by the strain-cultivar compatibility.

Based on common bunt infection levels, Armura and Pitar were the most susceptible cultivars to *Tilletia* spp. under the 2025-2026 field conditions, with mean BI values of 92.7% and 77.8%, respectively. According to previous evaluations conducted at NARDI Fundulea, these cultivars are classified as moderately susceptible (score 2) to moderately resistant (score 3) on a 1 to 5 resistance scale, where 1 indicates susceptibility and 5 indicates high resistance to the pathogen, under ecological conditions (Petcu, 2023).

The bacterial strains Ps.P and Ps.F showed the highest efficacy in suppressing disease development in FDL Columna and Armura wheat, although their protective effects were cultivar dependent (Table 4). In contrast, Ps.R showed limited efficiency against common bunt infection, except in Pitar cultivar. Among all treatments applied to this cultivar, Ps.R was the most effective and performed comparably to Ps.P strain, shown to be effective in other two cultivars also. Similar results were observed for the Ps.R and Ps.F treatments in the Izvor cultivar, which was the least responsive to the biocontrol treatments applied. The observed cultivar-dependent responses emphasize the importance of strain-cultivar compatibility in the successful implementation of biological control programs.

Among the tested bacterial isolates, Ps.P consistently provided the highest level of protection, reaching a maximum efficacy of 76.6% in FDL Columna variety. Ps.F also markedly reduced disease severity, particularly in Armura and FDL Columna, whereas Ps.R showed moderate overall performance but was comparatively more effective in Pitar.

Table 4. Biochemical traits of studied bacterial strains in experimental field conditions

Experimental variant	Outcome	Wheat variety			
		Armura	FDL Columna	Izvor	Pitar
Tilletia	BI (%)	92.7	46.2	53.9	77.8
Ps.F vs. *Tilletia*	BI (%)	43.2	26.0	44.7	57.1
	E (%)	53.4	43.7	17.1	26.6
Ps.P vs. *Tilletia*	BI (%)	42.4	10.8	47.6	44.1
	E (%)	54.3	76.6	11.7	43.3
Ps.R vs. *Tilletia*	BI (%)	74.2	37.0	44.5	42.3
	E (%)	20.0	19.9	17.4	45.6

The results further support previous reports regarding the potential of fluorescent and plant-associated pseudomonads as environmentally friendly alternatives to chemical seed treatments for common bunt management (McManus et al., 1993; Ali et al., 2014; Asthana et al., 2026; Wen et al., 2026).

Overall, the present study identifies Ps.P and Ps.F as promising candidates for the development of biological seed treatments against wheat bunt and contributes additional evidence supporting the integration of beneficial rhizobacteria into sustainable and organic wheat production systems.

CONCLUSIONS

This study demonstrated that the antagonistic potential of the investigated *Pseudomonas* strains is associated with a combination of antifungal and plant-beneficial traits, including the production of hydrolytic enzymes with antimicrobial effects, siderophore synthesis, and phosphate solubilization. The field experiments further revealed that the effectiveness of biological seed treatments against wheat common bunt is strongly influenced by cultivar-specific interactions, highlighting the importance of selecting compatible host genotype-microbial strain combinations for the development of reliable and sustainable biocontrol strategies.

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