



Isolation and Characterization of Native Fluorescent *Pseudomonas* Isolates from Uttarakhand Hills and Their Biocontrol Potential against Tomato Fusarium Wilt

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Pseudomonas spp. are recognized as efficient plant growth-promoting rhizobacteria (PGPR) with significant potential for the biological management of soil-borne phytopathogens. However, limited information is available regarding the diversity and biocontrol efficacy of native *Pseudomonas* isolates from the mid and high hill ecosystems of Uttarakhand. Therefore, the present investigation was undertaken during 2025–26 to isolate, characterize, and evaluate native fluorescent *Pseudomonas* isolates for their antagonistic potential against major soil-borne pathogens. A total of seventy rhizospheric soil samples were collected from seven

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districts of Uttarakhand covering altitudes ranging from 1370 to 3200 m above mean sea level. Twenty-three fluorescent *Pseudomonas* isolates were recovered on King's B medium and characterized through morphological and biochemical assays. All isolates exhibited positive reactions for catalase activity, KOH solubility, and ammonification tests, whereas twelve isolates showed starch hydrolysis activity. *In vitro* antagonistic evaluation through dual culture assay revealed considerable variability among isolates against *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, and *Sclerotium rolfsii*. Isolates PB1, PB4, and PS40 exhibited more than 75% inhibition of *F. oxysporum* f. sp. *lycopersici*, while PU2 showed maximum inhibition against *R. solani* (94.44%). Screening for biocontrol traits indicated that thirteen isolates produced hydrogen cyanide, whereas ten isolates exhibited phosphate solubilization and siderophore production. Under glasshouse conditions, seed biopriming with isolate PB1 significantly enhanced seed germination (93.33%) and reduced fusarium wilt disease incidence in tomato by 90% over the negative control. The study highlights the potential of native fluorescent *Pseudomonas* isolates, particularly PB1, as eco-friendly and sustainable biocontrol agents for the management of tomato Fusarium wilt and their possible future exploitation in integrated disease management programmes.

Keywords: Biocontrol; *Pseudomonas*; Rhizospheric soil; Antagonistic activity; Tomato wilt; *Fusarium oxysporum* f.sp. *lycopersici*.

1. Introduction

Beneficial microorganisms inhabiting the soil play a vital role in sustainable agriculture through improvement of soil health, enhancement of nutrient availability, and suppression of plant pathogens. Among these, plant growth-promoting rhizobacteria (PGPR) have attracted considerable attention owing to their ability to colonize the rhizosphere and promote plant growth through diverse mechanisms (de Sosa et al., 2023). PGPR enhance crop growth, productivity, and stress tolerance by facilitating nutrient uptake and through several direct and indirect mechanisms, including phosphate solubilization, siderophore production, biological nitrogen fixation, phytohormone production, antifungal activity, and induction of systemic resistance (Abd El-Mageed et al., 2022). Among the various PGPR, species belonging to the genus *Pseudomonas* constitute an important group of rhizobacteria recognized for their ability to stimulate plant growth and impart tolerance against both biotic and abiotic stresses through multiple mechanisms.

Pseudomonas spp., a group of ubiquitous Gram-negative, rod-shaped bacteria belonging to the family Pseudomonadaceae, are widely recognized for their effectiveness in the biological control of plant diseases (Kumaran et al., 2010). Fluorescent *Pseudomonas* spp. are known to solubilize phosphate and produce several hydrolytic enzymes such as amylase, protease, cellulase, and chitinase, along with important metabolites including hydrogen cyanide (HCN), siderophores, and indole acetic acid (Khalil et al., 2022). Members of this genus also secrete extracellular enzymes that help suppress pathogen invasion. Among the various antifungal metabolites produced, 2,4-diacetylphloroglucinol has been identified as a major factor contributing to the biocontrol potential of *Pseudomonas* spp. (Delany et al., 2000). In addition to pathogen suppression, the genus has been reported to enhance the growth and yield of several crops such as canola, wheat, rice, maize, soybean, cucumber, brinjal, and tomato (Cattelan et al., 1999) through mobilization of nutrients such as phosphorus and potassium into plant-available forms (Wang et al., 2020). In tomato, *Pseudomonas* spp. have been extensively investigated for their ability to manage both soil-borne and foliar pathogens, thereby improving plant health and crop productivity.

Solanum lycopersicum (tomato) is one of the most important solanaceous vegetable crops cultivated worldwide, ranking after potato and sweet potato in terms of area under cultivation, while also being the most extensively processed vegetable crop (Tamburino et al., 2020). Tomato is susceptible to several fungal diseases, including early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Septoria leaf spot (*Septoria lycopersici*), leaf mold (*Passalora fulva*), buckeye rot (*Phytophthora parasitica*), anthracnose (*Colletotrichum coccoides*), and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Ketelaar & Kumar, 2002). Among these, Fusarium wilt is considered one of the most destructive diseases of tomato, causing yield losses ranging from 10 to 100% (Haq & Ijaz, 2020). In India, the disease has been reported to cause yield losses of up to 47.94% (Choudhary et al., 2023). Management of the disease remains difficult because of the soil-borne nature and persistent survival ability of the pathogen. Conventional management strategies primarily rely on synthetic fungicides, which are often expensive, environmentally hazardous, and associated with risks to human health

(Jaiswal et al., 2017). Therefore, the development and adoption of eco-friendly and sustainable management approaches for tomato Fusarium wilt have become imperative. In this regard, seed biopriming has emerged as an effective and widely accepted strategy for enhancing plant health and tolerance against biotic stresses (Haruna et al., 2024).

Seed biopriming, defined as the treatment or coating of seeds with beneficial microorganisms (Mathre et al., 1999), has been recognized as an efficient approach for improving seed germination, seedling vigour, and tolerance to both biotic and abiotic stresses (Harman & Taylor, 1988). Furthermore, it enhances plant resistance against pathogens and improves overall plant performance under stress conditions (Rawat et al., 2016). In this context, bacterial biocontrol agents such as *Pseudomonas* spp. have gained considerable importance for disease management and plant growth promotion. Considering the immense potential of native rhizobacterial resources, the present investigation was undertaken to manage tomato Fusarium wilt in the hill regions of Uttarakhand through seed biopriming using effective fluorescent *Pseudomonas* isolates.

2. Material and Methods

The study was carried out in the Biological Control Laboratory of the Department of Plant Pathology, College of Hill Agriculture, Campus- Ranichauri, Tehri Garhwal, V.C.S.G., Uttarakhand University of Horticulture and Forestry, Uttarakhand.

2.1 Source of Materials (Soil, Pathogen, Plant Material) and Isolation of *Pseudomonas* spp.

2.1.1 Soil Samples

Survey and collection of rhizospheric soil samples was done from seven districts (viz., Tehri, Nainital, Pauri, Bageshwar, Uttarkashi, Almora and Chamoli) of Uttarakhand from various crops. Seventy rhizospheric soil samples were collected covering altitudes from 1370 m to 3200 m above mean sea level. The soil attached to secondary roots was gently removed using a brush, thoroughly mixed and about 10 g of composite soil was collected per field in properly labelled sample bags for further analysis.

2.1.2 Fungal Plant Pathogens

Fungal plant pathogens viz., *Fusarium oxysporum* f.sp. *lycopersici*, *Rhizoctonia solani* and *Sclerotium rolfsii*, were obtained from the microbial repository of the Department of Plant Pathology, College of Hill Agriculture, Campus- Ranichauri, Tehri Garhwal, V.C.S.G., Uttarakhand University of Horticulture and Forestry, Uttarakhand.

2.1.3 Seed Material

The seed material for the present investigation, comprised of the local tomato variety “S-21” (*Solanum lycopersicum*) was obtained from the local market of Ranichauri, Tehri Garhwal.

2.1.4 Isolation of *Pseudomonas* spp.

For the selective isolation of *Pseudomonas* isolates, 'serial dilution' technique was used on KB (King's B) medium plates followed by incubation at 28 ± 2 °C for 48 hrs. After incubation, plates were examined under UV light and colonies giving yellow-green fluorescence were isolated on Nutrient Agar Medium. Mother cultures were then maintained at 4 °C for further study.

2.2 In vitro Characterization of Isolated *Pseudomonas* Isolates

2.2.1 Morphological and Biochemical Characterization of Bacterial Isolates

The *Pseudomonas* isolates form characteristic colonies on the King's B medium, which was taken as a tool for preliminary identification. Cultural characterization of isolates was observed by different characteristics of colonies such as shape, nature, and colour pigmentation of the colony (Srinivasa et al., 2013). Biochemical

characterization of bacterial isolates was performed using various test like Gram's staining Catalase test, KOH test, Starch hydrolysis and Ammonification test (Bagul et al., 2023).

2.3 *In vitro* Evaluation for Biocontrol and Other Traits of *Pseudomonas* Isolates (Antagonistic Activity, HCN Production, Phosphate Solubilization and Siderophore Production)

2.3.1 Evaluation for Antagonistic Activity

In vitro antagonistic activity of *Pseudomonas* isolates against *R. solani*, *S. rolfsii* and *F. oxysporum* f.sp. *lycopersici* was studied using dual culture technique (Jishma et al., 2021). A three-to-four-day old culture of bacterial isolate was streaked at one side of petri dish (1 cm away from the edge) and 5 mm mycelial plug from three to four-day old cultures of pathogens were placed at the opposite side of the petri dish perpendicular to the bacterial streak on PDA medium. Petri dishes were then incubated at 28 ± 2 °C for 7 days. Petri dishes inoculated with sole fungal discs were served as a control. Each treatment was replicated five times. Observations on the width of mycelial growth of tested pathogens were recorded and the percent inhibition of pathogen growth was calculated using the following formula (Kamaruzzaman et al., 2021).

$$\text{The percent fungal growth inhibition was estimated by } I = \frac{C-T}{C} \times 100,$$

Where:

I = percent mycelial growth inhibition of the test pathogen.

C = mycelial growth of the pathogen in the control plate

T = mycelial growth of the pathogen in the test plate.

2.3.2 Evaluation for HCN Production, Phosphate Solubilization and Siderophore Production

The isolates were evaluated for biocontrol and plant growth-promoting traits, namely hydrogen cyanide (HCN) production, siderophore production, and phosphate solubilization. HCN production was determined by the modified method (Millar & Higgins, 1970) where a colour change from white to yellow-orange, brown, or brick red indicated a positive reaction. Siderophore production was assessed using the Chrome Azurol S (CAS) agar plate assay (Schwyn & Neilands, 1987) after incubation at 28 ± 2 °C for 72 hours, and the appearance of an orange halo zone confirmed the production. Phosphate solubilization was tested on Pikovskaya's agar medium (Linu et al., 2019) incubated at 28 ± 2 °C for 48 hours, where the formation of clear halo zone around bacterial colonies indicated tricalcium phosphate solubilization.

2.3.3 Developing Bioformulations of Promising *Pseudomonas* Isolates Exhibiting Broad Action Properties

Putative candidates of fluorescent *Pseudomonas* spp., PS1, PS2, PS10, PB1, and PB4, were selected for preparing talc-based bioformulation based on their preliminary screening & performance in the above studied traits. Sterilized King's broth was inoculated with the selected isolates separately and incubated at 28 ± 2 °C for 48–72 hrs on an incubator shaker at 150 rpm. (Sendhilvel et al., 2007). After 72 hrs, the inoculated broth was mixed with pre-sterilized raw talc (1:2 v/w) having 1.0 % carboxymethyl cellulose (CMC). The talc-based bioformulation was dried and the final CFU value was maintained at 10^{-7} g⁻¹. Talc-based bioformulation with calculated CFU value (Gupta & Dohroo, 2014) was packed in pre-autoclaved bags and stored at -20 °C till further use.

2.4 Evaluation of biocontrol Potential of Selected *Pseudomonas* Isolates against Tomato Wilt Disease Caused by *F. oxysporum* f. sp. *lycopersici* under Glass House Conditions

2.4.1 *Fusarium* Inoculum Preparation

Fresh Inoculum of *F. oxysporum* f. sp. *lycopersici* was prepared on sterilized barnyard millet grains and incubated at 28 °C for 12 days (Saber et al., 2015). Colonized grains were air-dried and grained to obtain a fine powder, which was passed through 80 mesh sieves to obtain a pure spore powder.

2.4.2 Pot Preparation

Soil was prepared by adding mixture of Soil: Sand: Farmyard manure (3:1:1), then transferred (5 kg/pot) into sterilized plastic pots (30 cm diameter). Infestation was done by thoroughly mixing 10 g of prepared fresh fungal inoculum per pot up to 5 cm depth and incubating under warm, humid conditions for eight days, except for T₁(Positive control) as described by Saber et al., (2015).

2.4.3 Seed Biopriming and Seed Sowing

Seeds were bioprimed with talc-based bioformulations of five putative fluorescent *Pseudomonas* isolates separately by incubating the treated seeds under warm and moist conditions until just prior to radicle emergence. Treatments T₁ (positive control) and T₂ (negative control) consisted of non-bioprimed seeds. Ten seeds were sown in each pot. The details of the treatments used in the present study is given in Table 1.

2.4.4 Observations

Percent Disease Incidence (PDI) was calculated, first after 30 days of sowing and was next calculated 60 days after sowing when all plants in T₂ (Negative control) wilted, by using the following formula (Shiva et al., 2020).

$$PDI \% = \frac{\text{No. of plants wilted}}{\text{Total number of plants examined}} \times 100$$

Percent Disease Reduction (PDR) over T₂ (Negative control) was recorded according to Alwathnani et al., (2012).

$$PDR \% = \frac{DIC - DIT}{DIC} \times 100$$

Where:

DIC = Disease incidence in Negative control

DIT = Disease incidence in treatment

2.4.5 Statistical Analysis

The replicated data of each treatment obtained in laboratory observations were statistically analysed using a Complete Randomised Design (CRD), using MS-Excel and OPSTAT. The mean value of the data was subjected to analysis of variance, as accepted by (Gomez & Gomez, 1984).

3. Results and Discussion

3.1 Isolation of *Pseudomonas* Isolates from Rhizospheric Soil Samples

Out of 70 soil samples tested, 23 soil samples harboured *Pseudomonas* isolates (Table 2). The number of *Pseudomonas* isolates isolated from soil samples varied across seven different districts. Maximum number of *Pseudomonas* isolates (six) were isolated from Tehri district followed by Nainital and Pauri district (four isolates from each). Similar approach for the isolation of *Pseudomonas* from rhizospheric soils has earlier been documented where the respective researchers successfully employed serial dilution technique, highlighting its reliability and widespread applicability in microbial studies (Suman et al., 2018; Joshi et al., 2018; Kipgen et al., 2021).

3.2 *In vitro* Characterization of Fluorescent *Pseudomonas* Isolates

3.2.1 Morphological and Biochemical Characterization

A total of 23 fluorescent *Pseudomonas* isolates were morphologically characterized on King's B medium and exhibited considerable variation in colony traits. All isolates produced characteristic fluorescence under UV

light (Fig. 1). Six bacterial isolates (PS1, PS32, PS40, PS43, PS45 and PU2) showed spreading type growth and remaining 17 showed non- spreading type growth (Fig. 2a). Colony colour ranged from cream white to white, pale yellow and white pink (Fig. 2b and Fig. 3). These observations are in agreement with the findings of Soesanto et al., (2010).

Further, biochemical characterization revealed that all isolates were Gram negative, rod shaped and tested positive for Catalase and KOH tests, indicating typical *Pseudomonas* characteristics (Table 4 and Fig. 4). In Starch hydrolysis test, 12 isolates exhibited positive results, and the rest 11 showed negative results. All the isolates showed positive results for Ammonification test (Table 4). These findings are consistent with earlier reports by Behrendt et al., (2003) and Hugh & Leifson (1953) confirming the identity and functional diversity of fluorescent *Pseudomonas* isolates.

3.2.2 *In vitro* Evaluation for Biocontrol and Other Traits of Fluorescent *Pseudomonas* Isolates (Antagonistic Activity, HCN Production, Phosphate Solubilization and Siderophore Production)

All 23 rhizobacterial isolates were evaluated for their antagonistic efficacy against major soil-borne fungal pathogens viz., *F. oxysporum* f. sp. *lycopersici*, *R. solani* and *S. rolfii* using the dual culture assay with four-days-old pathogen cultures, which revealed significant variability among fluorescent *Pseudomonas* isolates. Isolates PB1, PB4 and PS40 showed strong inhibition (>75%) against *F. oxysporum* f. sp. *lycopersici* while PU2 exhibited maximum inhibition (94.44%) against *R. solani*. PS40 also recorded high inhibition (86.67%) against *S. rolfii*, whereas PU1, PA1, PS45, PB4 and PS43 showed moderate inhibition (>50%) indicating their potential as effective biocontrol agents as shown in Table 3. These findings are in agreement with Singh et al., (2019) who earlier reported that *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* exhibited maximum mycelial inhibition against *F. oxysporum* and also suppressed *R. solani*. Further screening of plant growth-promoting traits revealed that 13 isolates showed positive results for hydrogen cyanide (HCN) production, indicated by a colour change from yellow to orange or brick red to brown with increasing intensity from day 4 to day 7 (Table 4 and Fig. 5).

In the phosphate solubilisation assay 10 isolates showed clear zone formation on Pikovskaya's agar (Fig. 6) and also produced siderophores on Chrome Azurol S medium (Table 4). These results are consistent with earlier findings by Kipgen et al., (2021) and Kamei and Simon (2018) indicating that HCN production, siderophore production and phosphate solubilisation contributes significantly to the antagonistic and plant growth-promoting potential of fluorescent *Pseudomonas* isolates.

3.2.3 Biocontrol Potential of *Pseudomonas* Isolates against Tomato Wilt Caused by *F. oxysporum* f. sp. *lycopersici* under Glass House Conditions

The antagonistic activity of putative *Pseudomonas* isolates against *F. oxysporum* f. sp. *lycopersici* in tomato was evaluated using selected *Pseudomonas* isolates (PS1, PS2, PB1, PS10, and PB4) applied through seed bioprimering. The bioprimered treatments performed significantly better than T₂ (Negative control) in terms of wilt suppression and seed germination. Germination ranged from 53.33% to 100% with maximum germination (100%) recorded in T₁ (Positive control), while Percent Disease Incidence ranged from 0% to 100% with the highest disease incidence in T₂ (Negative control). Among treatments, T₅ (*Fusarium* inoculated soil +Seeds bioprimered with PB1) recorded the highest Percent Disease Reduction (90%) over Negative control, indicating strong antagonistic efficiency (Table 5). The reduction in wilt may be attributed to the production of secondary metabolites such as siderophores, antibiotics, volatile compounds and lytic enzymes by *Pseudomonas* isolates. Similar findings were reported by Saraf et al., (2014) who demonstrated that *Pseudomonas* spp. inhibits phytopathogens by producing antimicrobial metabolites and detoxifying enzymes that degrade fungal cell walls and neutralize toxins. Shiva et al., (2020) observed significant suppression of tomato wilt caused by *F. oxysporum* f.sp. *lycopersici* where *Pseudomonas* isolates reduced disease incidence to 7.8% and achieved 85.6% control in artificially inoculated pots. These results confirm the potential of *Pseudomonas* isolates as effective biocontrol agents for managing *Fusarium* wilt disease through seed bioprimering in tomato.

Table 1. Details of treatments used in the present study to assess biocontrol potential of selected *Pseudomonas* isolates against tomato fusarium wilt

Symbol	Treatment
T ₁	Positive control (Untreated control): Non- <i>Fusarium</i> inoculated soil + Non bioprimered seeds
T ₂	Negative control: <i>Fusarium</i> inoculated soil+ Non bioprimered seeds
T ₃	<i>Fusarium</i> inoculated soil + Seeds bioprimered with <i>Pseudomonas</i> isolate PS1
T ₄	<i>Fusarium</i> inoculated soil + Seeds bioprimered with <i>Pseudomonas</i> isolate PS2
T ₅	<i>Fusarium</i> inoculated soil + Seeds bioprimered with <i>Pseudomonas</i> isolate PB1
T ₆	<i>Fusarium</i> inoculated soil + Seeds bioprimered with <i>Pseudomonas</i> isolate PS10
T ₇	<i>Fusarium</i> inoculated soil + Seeds bioprimered with <i>Pseudomonas</i> isolate PB4

Table 2. Details of the rhizospheric soil samples collected from mid and high hills of Uttarakhand

S. No.	Soil sample code	Crop	Location (Village)	District	Elevation (m above msl)	Harboured <i>Pseudomonas</i>	<i>Pseudomonas</i> isolate code
1	S1	Cabbage	Ranichauri	Tehri	1950	Yes	PS1
2	S2	Brinjal	Ranichauri	Tehri	1950	Yes	PS2
3	S3	Chilli	Ranichauri	Tehri	1950	No	-
4	S4	Capsicum	Ranichauri	Tehri	1950	No	-
5	S5	Potato	Salamkhet	Tehri	2300	No	-
6	S6	Rye	Salamkhet	Tehri	2300	No	-
7	S7	Rajma	Salamkhet	Tehri	2300	No	-
8	S8	Beans	Salamkhet	Tehri	2300	No	-
9	S9	Amaranth	Salamkhet	Tehri	2300	No	-
10	S10	Cabbage	Salamkhet	Tehri	2300	Yes	PS10
11	S11	Rajma	Ruichanu	Tehri	1930	No	-
12	S12	Maize	Ruichanu	Tehri	1930	No	-
13	S13	Barnyard	Ruichanu	Tehri	1930	No	-
14	S14	Finger millet	Ruichanu	Tehri	1930	Yes	PS14
15	S15	Rajma	Kainchu	Tehri	1918	No	-
16	S16	Barnyard	Kainchu	Tehri	1918	No	-
17	S17	Soybean	Kainchu	Tehri	1918	No	-
18	S18	Rajma	Kotdwara	Tehri	1948	No	-
19	S19	Barnyard	Kotdwara	Tehri	1931	No	-
20	S20	Maize	Kotdwara	Tehri	1931	No	-
21	S21	Tomato	Kotdwara	Tehri	1900	Yes	PS21
22	S22	Chilli	Kotdwara	Tehri	1900	No	-
23	S23	Amaranth	Kotdwara	Tehri	1880	No	-
24	S24	Rye	Maun	Tehri	1950	No	-
25	S25	Raddish	Maun	Tehri	1950	No	-
26	S26	Potato	Maun	Tehri	1950	No	-
27	S27	Barnyard	Maun	Tehri	1950	No	-
28	S28	Amaranth	Maun	Tehri	1900	No	-
29	S29	Chilli	Maun	Tehri	1900	Yes	PS29
30	S30	Rajma	Guriyali	Tehri	1868	No	-
31	DG	Pea	Guriyali	Tehri	1868	No	-
32	S32	Chilli	Pangot	Nainital	2250	Yes	PS32
33	S33	Okra	Pangot	Nainital	2250	Yes	PS33
34	S34	Maize	Sigri	Nainital	2200	No	-
35	S35	Turmeric	Sigri	Nainital	2200	Yes	PS35
36	S36	Pumpkin	Sigri	Nainital	2200	No	-
37	S37	Bitter gourd	Sattal	Nainital	1370	Yes	PS37
39	S38	Sponge gourd	Sattal	Nainital	1370	No	-
40	S39	Cucumber	Sattal	Nainital	1370	No	-
41	S40	Barnyard millet	Kaproli	Pauri	2200	Yes	PS40
42	S41	Pumpkin	Kaproli	Pauri	2180	No	-
43	S42	Maize	Kaproli	Pauri	2180	Yes	PS42
44	S43	Rice	Kaproli	Pauri	2150	Yes	PS43
45	S44	Finger millet	Mursheti	Pauri	1770	No	-
46	S45	Amaranth	Mursheti	Pauri	1800	Yes	PS45

S. No.	Soil sample code	Crop	Location (Village)	District	Elevation (m above msl)	Harboured <i>Pseudomonas</i>	<i>Pseudomonas</i> isolate code
47	S46	Urad	Mursheti	Pauri	1800	No	-
48	S47	Amaranth	Buranshi	Pauri	1650	No	-
49	S48	Maize	Buranshi	Pauri	1700	No	-
50	B1	Soybean	Kafligair	Bageshwar	1245	Yes	PB1
51	B2	Colocasia	Kafligair	Bageshwar	1245	No	-
52	B3	Barnyard	Bharadi	Bageshwar	1522	No	-
53	B4	Ginger	Vijaypur	Bageshwar	1530	Yes	PB4
54	B5	Marigold	Vijaypur	Bageshwar	1530	No	-
55	B6	Raddish	Vijaypur	Bageshwar	1520	No	-
56	B7	Urad	Sama	Bageshwar	2500	No	-
57	U1	Soybean	Purola	Uttarkashi	1500	Yes	PU1
58	U2	Rajma	Purola	Uttarkashi	1500	Yes	PU2
59	U3	Beefsteak plant	Mainjni	Uttarkashi	2059	No	-
60	A1	Beans	Kausani	Almora	1890	Yes	PA1
61	A2	Cabbage	Kausani	Almora	1890	No	-
62	A3	Summer squash	Ranikhet	Almora	1869	No	-
63	A4	Pumpkin	Ranikhet	Almora	1869	No	-
64	A5	Cucumber	Jageshwar	Almora	1870	Yes	PA5
65	CH1	Torai	Bhadura	Chamoli	1500	No	-
66	CH2	Pumpkin	Bhadura	Chamoli	1500	Yes	PCH2
67	CH3	Brinjal	Malari	Chamoli	3050	Yes	PCH3
68	CH4	Cucumber	Malari	Chamoli	3050	No	-
69	CH5	Okra	Mana	Chamoli	3200	Yes	PCH5
70	CH6	Bottle gourd	Mana	Chamoli	3200	No	-

Table 3. Antagonistic activity and performance of fluorescent *Pseudomonas* isolates against *F. oxysporum* f. sp. *lycopersici*, *R. solani* and *S. rolfisii*

S. No.	<i>Pseudomonas</i> isolate code	% Inhibition of <i>F. oxysporum</i> f. sp. <i>lycopersici</i>	% Inhibition of <i>R. solani</i>	% Inhibition of <i>S. rolfisii</i>	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	<i>R. solani</i>	<i>S. rolfisii</i>
1	PS1	59.26*± 0.98	55.19*± 0.37	19.26*± 1.61	++ve	++ ve	+ ve
2	PS2	67.04*± 0.98	55.93*± 0.37	13.33*± 1.11	++ve	++ ve	+ ve
3	PB1	75.93*± 0.98	77.04*± 0.74	17.41*± 0.74	+++ ve	+++ ve	+ ve
4	PS10	29.63*± 0.98	44.82*± 0.37	31.85*± 0.98	+ ve	++ ve	++ ve
5	PS14	42.59*± 0.98	50.00*± 1.28	22.22*± 1.28	++ ve	++ ve	+ ve
6	PB4	79.26*± 0.74	87.04*± 0.98	55.19*± 0.98	+++ ve	+++ ve	++ ve
7	PS21	10.37*± 0.74	61.48*± 1.61	41.11*± 1.28	+ ve	++ ve	++ ve
8	PS29	12.22*± 0.64	57.41*± 0.37	33.33*± 1.28	+ ve	++ ve	++ ve
9	PS32	36.67*± 0.64	56.30*± 0.74	11.85*± 0.74	++ ve	++ ve	+ ve
10	PS33	32.59*± 0.74	60.74*± 0.98	15.93*± 0.74	++ ve	++ ve	+ ve
11	PS35	65.56*± 0.64	51.11*± 0.64	13.33*± 1.28	++ ve	++ ve	+ ve
12	PS37	42.59*± 0.98	71.48*± 0.74	31.85*± 0.98	++ ve	+++ ve	++ ve
13	PS40	77.04*± 0.74	54.82*± 0.74	86.67*± 1.70	+++ ve	++ ve	+++ ve
14	PS42	49.26*± 0.74	52.22*± 1.28	21.85*± 0.98	++ ve	++ ve	+ ve
15	PS43	67.04*± 0.37	53.33*± 1.28	53.33*± 1.70	++ ve	++ ve	++ ve
16	PS45	32.96*± 0.37	50.00*± 1.70	64.07*± 1.61	++ ve	++ ve	++ ve
17	PU1	51.85*± 0.98	56.67*± 1.11	77.04*± 0.74	++ ve	++ ve	+++ ve
18	PU2	67.04*± 0.98	94.44*± 3.21	21.11*± 0.64	++ ve	+++ ve	+ ve
19	PA1	11.85*± 0.74	52.22*± 1.11	75.56*± 1.28	+ ve	++ ve	+++ ve
20	PA5	37.04*± 0.98	60.74*± 0.98	34.07*± 0.74	++ ve	++ ve	++ ve
21	PCH2	66.30*± 0.37	75.56*± 1.28	13.33*± 1.28	++ ve	+++ ve	+ ve
22	PCH3	60.37*± 0.74	65.19*± 0.98	26.30*± 0.98	++ ve	++ ve	+ ve
23	PCH5	11.85*± 0.74	52.59*± 1.61	42.59*± 0.98	+ ve	++ ve	++ ve
24	Control	0.00± 0.00	0.00± 0.00	0.00± 0.00			
	SE (d)	1.11	1.69	1.61			
	C.D(0.05)	2.23	3.42	3.24			

Key words: + <30 %, ++ 30-70 %, +++ >70 %, *Significant at 5% level of significance as compared to Control

Table 4. Performance of fluorescent *Pseudomonas* isolates towards different biochemical tests (Gram's staining, catalase test, KOH test, starch hydrolysis, and ammonification test), along with evaluation for biocontrol traits including HCN production, phosphate solubilization, and siderophore production

S. No.	<i>Pseudomonas</i> isolate code	Biochemical Tests					Biocontrol Traits		
		Gram's staining	Catalase test	KOH test	Starch hydrolysis	Ammonification test	HCN production	Phosphate solubilisation	Siderophore production
1	PS1	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
2	PS2	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	PB1	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
4	PS10	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
5	PS14	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
6	PB4	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
7	PS21	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
8	PS29	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
9	PS32	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve
10	PS33	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
11	PS35	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve
12	PS37	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
13	PS40	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
14	PS42	-ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve
15	PS43	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
16	PS45	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
17	PU1	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
18	PU2	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
19	PA1	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
20	PA5	-ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
21	PCH2	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
22	PCH3	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
23	PCH5	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve

+ = Positive reaction, - = Negative reaction

Table 5. Evaluation of *Pseudomonas* isolates applied through seed biopriming for wilt disease suppression in tomato caused by *Fusarium oxysporium* f. sp. *lycopersici* under glass house conditions

S. No.	Treatments	Germination (%)	PDI (%)		PDR (%)
			30 DAS	**60 DAS	
1	T ₁	100.00*± 0.00	0.00± 0.00	0.00± 0.00	100.00*± 0.00
2	T ₂	53.33*± 3.33	60.00*± 5.57	100.00*± 0.00	-
3	T ₃	76.67*± 3.33	20.00*± 0.00	30.00*± 0.00	70.00*± 0.00
4	T ₄	83.33*± 3.33	23.33*± 3.33	26.67*± 3.33	73.33*± 3.33
5	T ₅	93.33*± 3.33	06.67*± 3.33	10.00*± 0.00	90.00*± 0.00
6	T ₆	70.00*± 0.00	16.67*± 3.33	33.33*± 3.33	66.67*± 3.33
7	T ₇	86.67*± 3.33	13.33*± 6.66	16.67*± 3.33	83.33*± 3.33
SE(d)		3.98	5.63	3.09	3.09
C.D _(0.05)		8.63	12.20	6.68	6.68

Where, PDI = Percent Disease Incidence, PDR = Percent Disease Reduction over Negative control (T₂)

*Significant at 5% level of significance

DAS=Days after sowing

**60 DAS= All plants in T₂ (Negative control) wilted/died

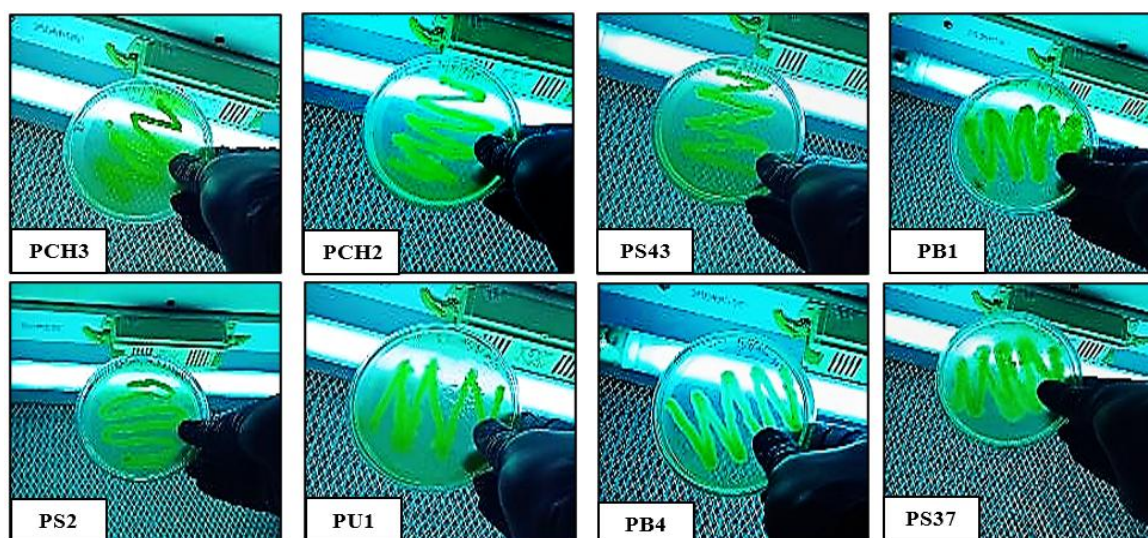


Fig. 1. Bacterial isolates exhibiting green fluorescence under UV light on King's B medium

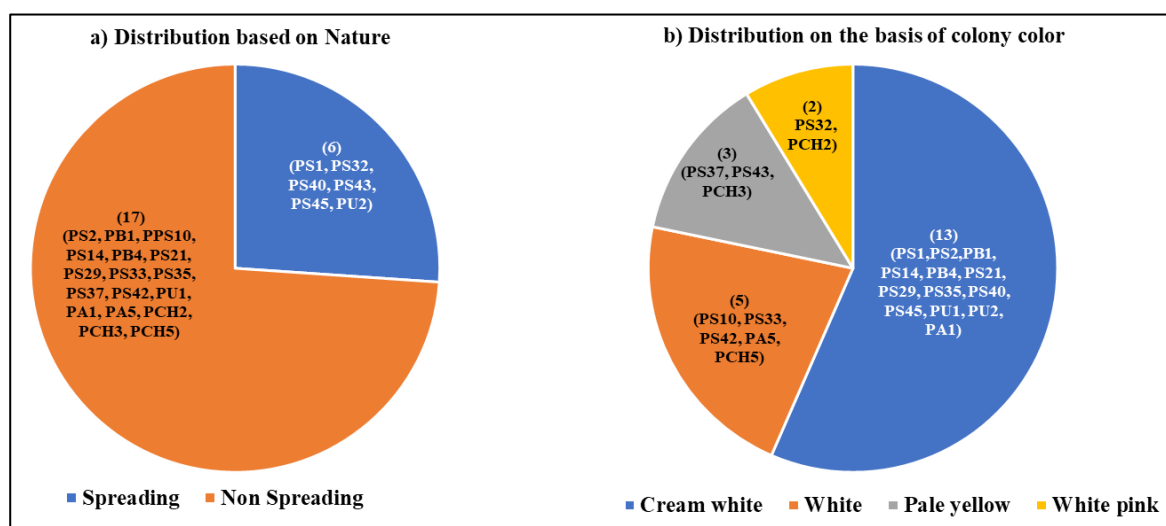


Fig. 2. Morphological characteristics of *Pseudomonas* isolates; a) Distribution based on spreading and non-spreading type; b) Distribution based on colony colour

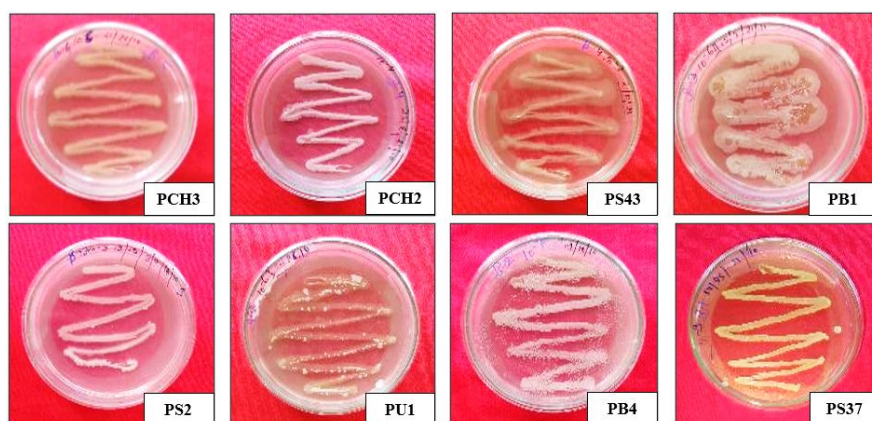


Fig. 3 Pure culture of *Pseudomonas* isolates producing slimy, white, cream white, pale yellow and white pink colonies on King's B medium.

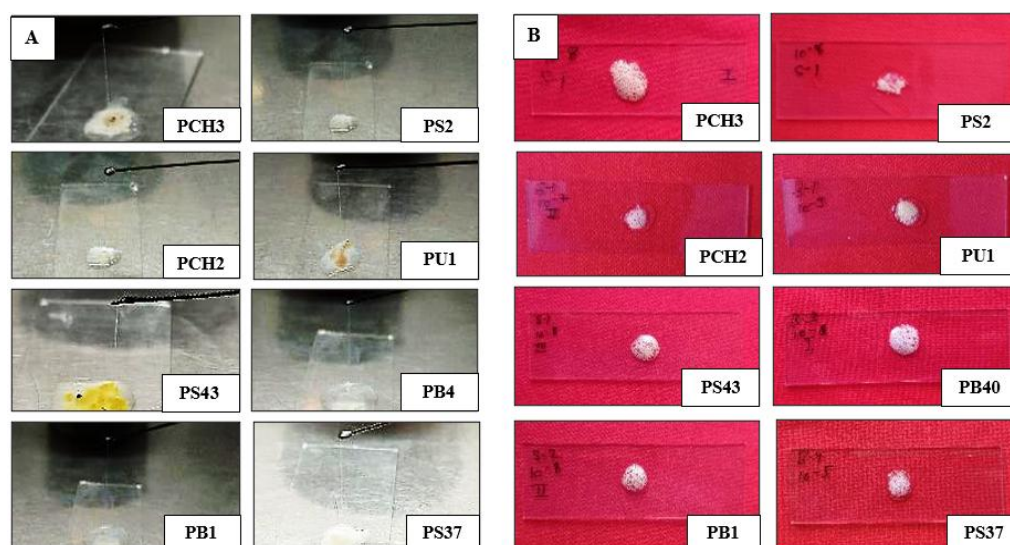


Fig. 4 Biochemical characterization of fluorescent *Pseudomonas* isolates; (A) KOH test, (B) Catalase test

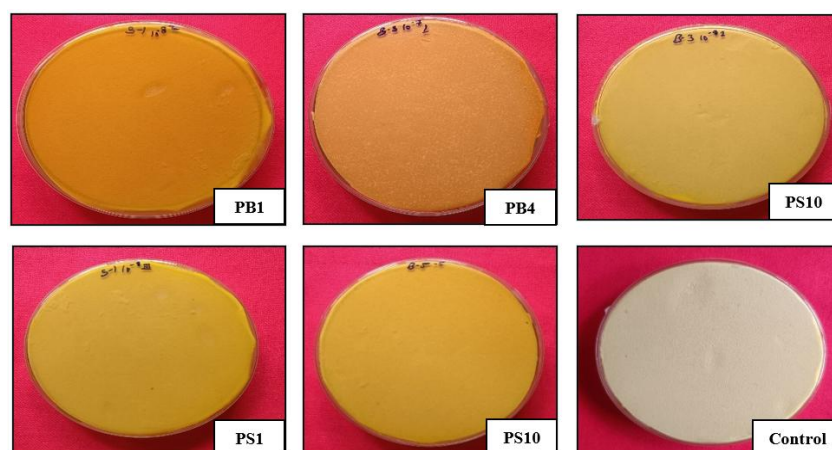


Fig. 5 Evaluation of fluorescent *Pseudomonas* isolates for HCN production. Change of white coloured filter paper from yellow to orange indicates positive reaction

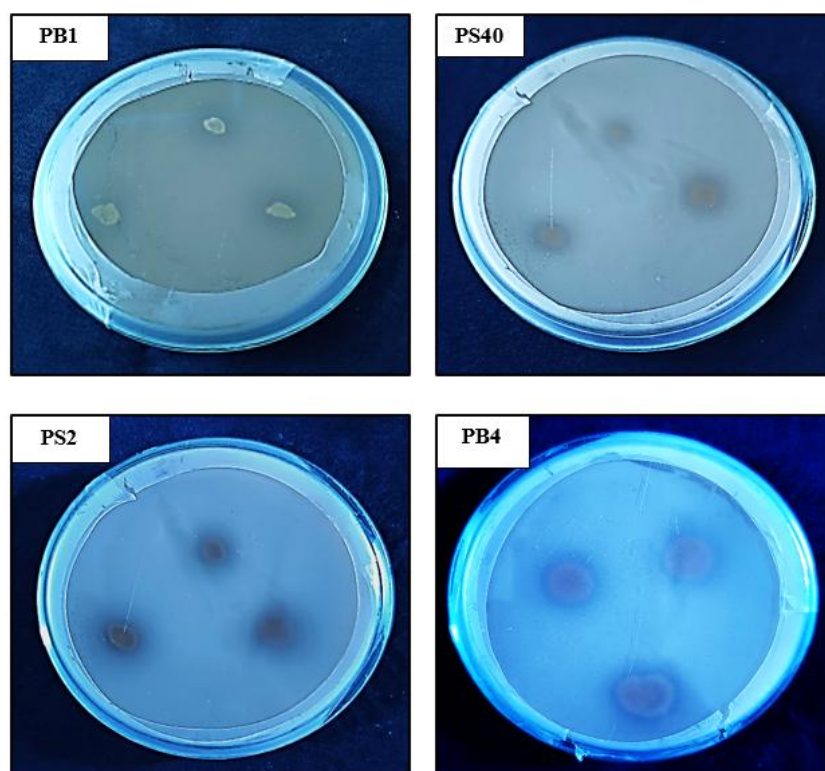


Fig. 6. Evaluation of fluorescent *Pseudomonas* isolates for phosphate solubilization on Pikovskaya's agar medium. A clear zone of solubilization around the bacterial growth indicates positive response

4. Conclusion

The present investigation demonstrated the immense biocontrol potential and functional diversity of native fluorescent *Pseudomonas* isolates recovered from the rhizosphere of crops grown in the mid and high hill regions of Uttarakhand. The study successfully established that several isolates possessed strong antagonistic activity against major soil-borne fungal pathogens, namely *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, and *Sclerotium rolfsii*, along with important plant growth-promoting and biocontrol attributes such as hydrogen cyanide production, phosphate solubilization, siderophore production, catalase activity, and ammonification. Among the evaluated isolates, PB1 emerged as the most promising candidate, exhibiting superior suppression of tomato Fusarium wilt under glasshouse conditions when applied through seed biopriming. The substantial reduction in disease incidence coupled with enhanced seed germination clearly indicates its potential role in promoting early plant establishment and suppressing pathogen development in a sustainable manner.

The findings of the present study provide valuable insights into the unexplored microbial diversity of Uttarakhand hill ecosystems and emphasize the importance of native rhizobacterial resources in developing environmentally safe alternatives to chemical fungicides. The identified isolates, particularly PB1, may serve as potential candidates for the development of bioformulations and integrated disease management strategies in tomato cultivation. Furthermore, this work lays a scientific foundation for future molecular characterization, metabolite profiling, formulation development, and multilocational field evaluation of promising isolates for their commercial exploitation in sustainable agriculture and biological disease management programmes.

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Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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