



Unveiling broad-spectrum microbial antagonists for sustainable management of seed-borne diseases of lentil (*Lens culinaris*)

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ABSTRACT

Lentil (*Lens culinaris* Medik) productivity is significantly affected by seed-borne pathogens such as *Fusarium oxysporum* f.sp. *lentis*, *Rhizoctonia solani*, and *Botrytis cinerea*. The use of indigenous rhizospheric microbes as broad-spectrum biocontrol agents offers a sustainable strategy for disease management. The present investigation was conducted to isolate, characterise, and identify indigenous rhizospheric microorganisms possessing biocontrol and plant growth promoting (PGP) attributes. Forty fungal and thirty bacterial strains were isolated from lentil rhizosphere soil collected during winter (*rabi*) season of 2023–24 at ICAR-Indian Agricultural Research Institute, New Delhi, and screened for antagonist activity against the targeted pathogens employing a dual culture assay and biochemical traits. Among them, three fungal (F11, F16, F27) and two bacterial (B2, B13) isolates exhibited the strong and consistent inhibition of all pathogens. Morphological and cultural characterisation revealed typical features of potential antagonists, which were validated by molecular identification. Internal transcribed spacer (ITS) region sequencing identified the fungal isolates as *Trichoderma harzianum* (F11), *Trichoderma asperellum* (F16), and *Talaromyces flavus* (F27), whereas the bacterial isolates were identified through 16S rDNA sequencing as *Pseudomonas fluorescens* (B2) and *Bacillus subtilis* (B13). Phylogenetic analysis validated their taxonomic affiliation. Furthermore, the isolates exhibited multiple biocontrol and PGP traits, including hydrolytic enzymes, siderophores, hydrogen cyanide (HCN), ammonia, phosphate solubilisation, and indole-3-acetic acid (IAA). The combined expression of antagonistic and PGP activities highlights the potential of these indigenous microbial isolates as promising candidates for the development of eco-friendly biocontrol formulations, providing a strong basis for further evaluation under greenhouse and field conditions for integrated management of lentil diseases.

Keywords: Bioagents, Biocontrol, Characterisation, Identification, Rhizospheric-microbes

Grain legumes play a vital role in human nutrition, particularly in developing countries. Lentil (*Lens culinaris* Medik) is an important *rabi* season pulse crop, rich in protein (24–28%), carbohydrates (57–60%), essential amino acids (lysine), and minerals, making it vital for food and nutritional security in plant-based diets. Globally, lentil is cultivated over approximately 5.6 mha, with a total production of 7.06 mt and an average productivity of 1,245 kg/ha (FAO 2025). India ranks first in cultivated area (1.63 mha) and third in production (1.56 mt). It is the second-most important winter pulse after chickpea, primarily grown in the Northern, Central, and Eastern regions with Madhya Pradesh (6.25 lakh tonnes) as the leading producer, followed

by Uttar Pradesh (6.02 lakh tonnes) (Indiastat 2024). However, lentil production is constrained by several biotic stresses, including fungal, bacterial, and viral diseases, as well as parasitic weeds (Dita *et al.* 2006). Major diseases include wilt (*Fusarium oxysporum* f. sp. *lentis*), Alternaria blight, anthracnose, Ascochyta blight, grey mold (*Botrytis cinerea*), and root rot (*Rhizoctonia solani*), which cause yield losses (20–25%) depending on severity and environment (Jiskani *et al.* 2021). These diseases also deteriorate seed quality; Fusarium wilt, dry root rot, and grey mold are major threats, causing seedling death, reduced nodulation, and wilting without vascular discolouration (Singh 2017). Disease incidence is prevalent during the flowering and pod formation stages with up to 60–70% wilt (Garkoti *et al.* 2014).

Although broad-spectrum fungicides are effective, their overuse leads to disrupting microbial communities, harming ecosystems, increasing fungicide resistance, and health risks due to the accumulation of carcinogenic chemical

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residues in food (Rani *et al.* 2021). Bio-antagonists offers an effective alternative in minimising synthetic fungicides in agriculture. Owing to the importance of bioagents in disease management, the present investigation aimed to isolate, characterise, and identify potential bioagents and evaluate their antagonistic potential against diseases, including Fusarium wilt, dry root rot, and grey mold of lentil.

MATERIALS AND METHODS

Collection and maintenance of fungal pathogens: Single spore-derived pure cultures of fungal pathogens, namely *Fusarium oxysporum* f. sp. *lentis* (FOL), *Rhizoctonia solani* (RS), and *Botrytis cinerea* (BC) were obtained from the Indian Type Culture Collection (ITCC), ICAR-Indian Agricultural Research Institute, New Delhi. The cultures were sub-cultured on Potato Dextrose Agar (PDA) medium under aseptic conditions and preserved at 4°C for further use.

Isolation of rhizospheric bio-agents: Rhizosphere soil samples were collected from healthy lentil plants during the *rabi* season of 2023–24 and soil adhering to the root zone (5–10 cm depth) were collected using sterile blades into sterile polythene bags. Serial dilution and plating technique were followed, and PDA supplemented with streptomycin (50 µg/mL) for fungi, nutrient agar for bacteria, and King's medium B base specific for *Pseudomonas fluorescence* were used as basic medium. Plates were kept at 28 ± 2°C for 48–72 h for bacterial growth and 5–7 days for fungal growth, and distinct colonies were purified and maintained on respective agar slants for further characterisation.

Dual culture assay of isolated fungi and bacteria for antagonistic activity against *Fusarium oxysporum* f. sp. *lentis*, *Rhizoctonia solani*, and *Botrytis cinerea*: Antagonistic activity against *F. oxysporum* f. sp. *lentis*, *R. solani*, and *B. cinerea* was evaluated using dual culture assay (Haider *et al.* 2016). Fungal isolates were paired with pathogens on PDA, while bacterial isolates were streaked opposite a central pathogen disc on PDA+NA (3:1). After 5 days at 28 ± 2°C, pathogen radial growth was measured and compared with control plates. The percent inhibition was calculated using the given formula:

$$\text{Percentage inhibition (\%)} = \frac{\text{Control diameter} - \text{Test antagonist diameter}}{\text{Control diameter}} \times 100$$

Characterisation of selected microbial isolates for their biocontrol and plant growth-promoting (PGP) traits: Selected microbial isolates were characterised for their biocontrol mechanisms and PGP traits, including production of ammonia, siderophores, hydrogen cyanide (HCN), phosphorus solubilisation, indole-3-acetic acid (IAA), and hydrolytic enzymes (cellulase, chitinase, chitosanase, and β-1,3-glucanase). Siderophore production was detected using the chrome azurol S (CAS) agar assay (Schwyn and Neilands 1987). Isolates were spot-inoculated on CAS plates and incubated at 28 ± 2°C for 7 days. Formation of yellow to orange halos due to decolourisation of the blue CAS-Fe³⁺ complex indicated positive siderophore production. HCN

production was assessed following Bakker and Schippers (1987). Isolates were grown on glycine-supplemented medium (4.4 g/L) with filter paper soaked in 0.5% picric acid in 2% sodium carbonate placed in the lid. After incubation at 28 ± 2°C for 5–7 days, a colour changed from yellow to orange-brown confirmed HCN production. IAA production was evaluated by inoculating cultures in ISP-2 broth with and without tryptophan (10 mg/mL) (Ndeddy Aka and Babalola 2016). After 7 days of incubation with shaking at 28 ± 2°C, cultures were centrifuged (5000 ×g, 15 min). Supernatant reacted with orthophosphoric acid and Salkowsky reagent developed a pink colour, indicating IAA synthesis.

Phosphate solubilisation was assessed by spot inoculating isolates on Pikovskaya's agar and incubating at 30°C for 7 days; clear halo zones indicated positive activity (Pikovskaya *et al.* 1948). Ammonia production was tested by inoculating isolates in peptone water (5 mL) and incubating at 28 ± 2°C for 7 days. Nessler's reagent (0.5 mL) was added, and the appearance of yellow to deep pinkish-red colouration indicated ammonia production.

Hydrolytic enzyme activity (chitinase, chitosanase, β-1,3-glucanase, and cellulase) was evaluated using a well diffusion assay. Bacterial and fungal cultures were grown in substrate-amended broth (1%) at 30°C with shaking (150 rpm) for 72 h (bacteria) and 5 days (fungi). After centrifugation and filtration, 100 µL supernatant was loaded into 9 mm wells on substrate agar plates. Clear zones after 24 h at 37°C indicated enzyme activity. All the bioassay studies was replicated thrice and the experiment was repeated twice.

Morphological and biochemical characterisation of selected antagonists: Selected bacterial antagonists were studied for their morphological characteristics by colony characteristics and biochemical tests such as catalase test (Blezevic and Elderer 1975), potassium hydroxide test, lipase test, and Gram's reaction. Similarly, morphological and microscopic observation of fungal antagonists, including colony colour, pattern, pigmentation, sporulation time, spore morphology, and fruiting bodies, was also determined.

Molecular identification of potential antagonists using 16S rDNA and ITS gene sequencing: The three fungal and two bacterial antagonists, based on their *in vitro* antagonistic and PGP potentials, were selected for molecular identification through the Internal Transcribed Spacer (ITS) for fungi and 16S rDNA gene sequencing for bacteria. Genomic DNA of fungal isolate was extracted through the CTAB method, while bacterial DNA was extracted using a commercial bacterial genomic DNA extraction kit (QIAGEN DNA, Germany). The ITS region of fungi was amplified using universal primers; ITS 1 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3'), while the 16S rRNA gene of bacteria was amplified using primers 8F (5'-AGAGTTTGATCCTTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Kavya *et al.* 2025). PCR amplification was performed in a 25 µL reaction volume. For fungi, a Bio-Rad T100™ Thermal Cycler was used with an initial denaturation at 94°C for 4 min; 35 cycles of 94°C for 30 s, 56°C for 1 min, and 72°C for

1 min; followed by a final extension at 72°C for 10 min. For bacteria, amplification was carried out in a HiMedia thermal cycler with an initial denaturation at 95°C for 5 min; 35 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1.5 min; followed by a final extension at 72°C for 7 min. The amplified products were confirmed by agarose gel electrophoresis and sequenced using the Sanger method. Sequences were analysed using BLASTn in the NCBI GenBank database. Sequences alignment was performed using MUSCLE in MEGA 11.0, and phylogenetic trees were constructed using the Maximum Likelihood method with the Tamura-Nei model and 1,000 bootstrap replications (Tamura *et al.* 2021). The generated sequences were submitted in the NCBI GenBank database.

Statistical analysis: The data generated were analysed to determine the efficacy and range of various isolates in controlled environments using a completely randomised design (CRD). A two-way ANOVA was performed using GRAPES software (version 1.1.0) to assess the impact of local isolates and pathogen combinations, including their interaction. The post hoc Tukey's HSD (honestly significant difference) test was performed to determine mean separation, and significance was established at $p < 0.05$. To facilitate visualisation of the broad-spectrum isolates, a heat map was created in RStudio (version 0.92) using the pheatmap package.

RESULTS AND DISCUSSION

Screening of isolated microbes for broad-spectrum antagonistic traits against pathogens via dual culture assay: A group of 40 fungi and 30 bacteria were isolated from rhizosphere soil and screened against three pathogens, viz. *F.*

oxysporum f. sp. *lentis* (FOL), *R. solani* (RS), and *B. cinerea* (BC). *In vitro* assay showed distinct degrees of inhibition of FOL, RS, and BC. Fifteen fungal isolates showed effective antagonistic activity (>50% inhibition) against at least one pathogen. However, only three fungal isolates (F11, F16, and F27) showed broad-spectrum antagonistic activity against each of the three pathogens (Table 1, Fig. 2A). Among 30 bacterial antagonists, nine were effective against at least one pathogen, while only two isolates (B2 and B13) inhibited all three pathogens (Fig. 1, Fig. 2B). Isolate F11 significantly inhibited the radial mycelial growth of FOL from 68 mm (control) to 18 mm (73.5% inhibition), RS to 22.3 mm (72.1%) and BC to 21.0 mm, (72.0%). Isolate B2 demonstrated even stronger antagonistic effect, suppressing the growth of FOL, RS, and BC to 13 mm, 20.1 mm, and 19.9 mm, resulting in inhibition rates of 80.82%, 74.84%, and 73.45%, respectively (Fig. 2B). Other notable fungal isolates, including F16 (73.0%, 73.8%, and 68.4%) and F27 (65.7%, 67.9%, and 66.2%), showed broad-spectrum inhibition against FOL, RS, and BC, respectively (Fig. 2A). Additionally, the bacterial isolate B13 showed 72.9%, 72.9% and 71.1% against FOL, RS, and BC, respectively (Fig. 2B). Several isolates exhibited pathogen-specific antagonism (>50% inhibition), including multiple fungal and bacterial isolates against FOL, RS, or BC individually (Fig. 2). Similar results were found by Azeem *et al.* (2022), that states, out of 113 isolates screened, two bacterial antagonists (M11 and M33) from the maize phyllosphere had broad-spectrum antifungal activity against four phytopathogenic fungi (*Alternaria alternata*, *F. oxysporum* f. sp. *lentis*, *R. solani*, and *F. verticillioides*). Additionally, 10 bacterial isolates showed antagonistic

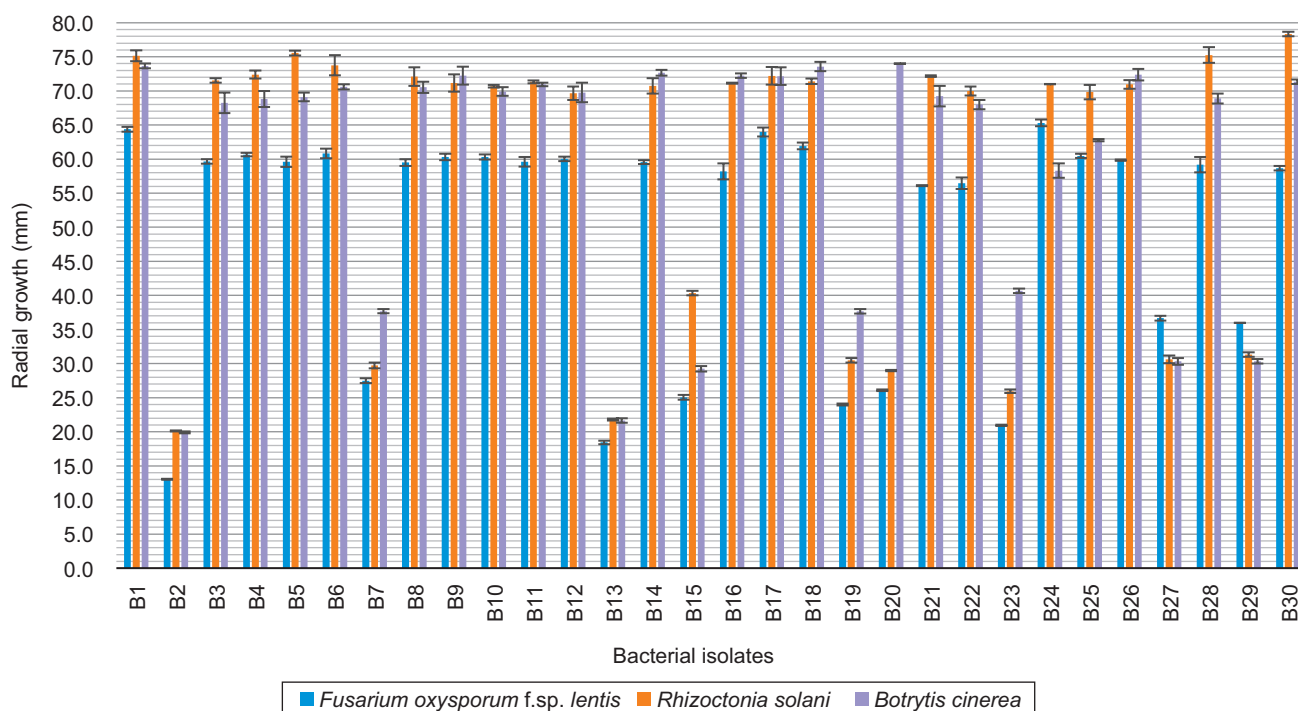


Fig. 1 Effect of bacterial antagonists on radial growth (mm) of pathogens (*F. oxysporum* f. sp. *lentis*, *R. solani*, and *B. cinerea*) under *in vitro*.

Table 1 *In vitro* efficacy of different fungal antagonists against FOL, RS and BC on radial growth via dual culture technique

Isolate/pathogen	FOL	RS	BC
Control	68.0 ± 0.14 ghij	79.9 ± 0.20 ^a	75.0 ± 0.12 ^c
F1	21.7 ± 0.30 345	22.0 ± 0.05 234	55.7 ± 0.85 EF
F2	51.9 ± 4.10 H	72.3 ± 0.85 de	68.1 ± 0.15 ghij
F3	52.3 ± 3.05 H	69.3 ± 0.36 fg	66.4 ± 0.45 jklmn
F4	53.3 ± 2.08 GH	58.3 ± 1.79 CD	66.1 ± 1.22 klmno
F5	19.7 ± 1.15 678	42.4 ± 0.57 KLM	37.8 ± 0.25 QR
F6	31.3 ± 0.737 U	42.7 ± 0.94 KLM	41.0 ± 1.00 MNO
F7	53.3 ± 1.15 GH	64.3 ± 1.15 opqrst	68.7 ± 0.74 ghi
F8	52.5 ± 1.32 GH	62.3 ± 0.57 uvwxy	58.8 ± 1.85 BC
F9	56.6 ± 1.64 DE	63.7 ± 0.57 qrstuv	61.4 ± 0.51 xyzA
F10	19.1 ± 1.50 78	65.3 ± 0.57 lmnopq	38.9 ± 0.32 PQ
F11	18.0 ± 1.05 ⁸	22.3 ± 2.08 1234	21.0 ± 1.00 456
F12	60.3 ± 0.57 zAB	68.9 ± 1.01 ghi	60.4 ± 1.11 zAB
F13	56.3 ± 0.57 ^E	68.1 ± 1.81 ghij	61.7 ± 0.40 wxyz
F14	24.0 ± 0.10 YZI	41.3 ± 1.15 LMNO	37.7 ± 0.58 QR
F15	21.3 ± 0.30 456	34.3 ± 0.57 ^T	43.0 ± 1.00 KL
F16	18.3 ± 0.57 78	21.0 ± 1.00 456	23.7 ± 1.15 ^{MNO}
F17	49.4 ± 0.90 ^I	63.0 ± 0.10 stuvwx	64.9 ± 1.30 mnopqr
F18	26.0 ± 0.25 ^X	39.7 ± 0.57 OP	39.7 ± 0.58 OP
F19	52.6 ± 0.95 GH	71.4 ± 2.27 ^c	65.8 ± 0.20 klmnop
F20	47.1 ± 0.26 ^J	68.8 ± 0.20 ghi	72.2 ± 1.32 de
F21	20.0 ± 0.25 567	36.7 ± 1.15 RS	38.3 ± 1.15 PQR
F22	52.0 ± 0.00 H	69.3 ± 0.94 fg	68.1 ± 0.85 ghij
F23	52.4 ± 1.09 H	71.1 ± 1.36 ef	63.3 ± 0.76 rstuvw
F24	54.3 ± 0.87 FG	58.8 ± 0.96 BC	64.6 ± 1.05 nopqrs

Isolate/pathogen	FOL	RS	BC
F25	55.7 ± 2.08 EF	63.8 ± 0.72 qrstu	66.0 ± 0.00 klmno
F26	49.2 ± 2.06 ^I	67.1 ± 1.27 ijkl	61.2 ± 1.40 yzA
F27	23.3 ± 0.57 123	25.7 ± 0.57 XY	25.3 ± 2.09 XYZ
F28	49.9 ± 0.80 ^I	69.1 ± 1.22 gh	59.8 ± 0.32 ABC
F29	58.3 ± 0.57 CD	73.8 ± 0.25 cd	67.3 ± 0.41 ^{hijk}
F30	20.0 ± 0.05 567	29.3 ± 1.15 ^V	40.1 ± 0.55 NOP
F31	29.1 ± 1.15 ^V	26.1 ± 0.15 WX	38.6 ± 0.83 PQ
F32	52.7 ± 1.52 GH	76.9 ± 2.43 ^b	68.1 ± 1.20 ghij
F33	52.7 ± 2.30 GH	73.5 ± 1.32 cd	67.4 ± 0.40 ^{hijk}
F34	46.3 ± 0.52 ^J	35.2 ± 0.20 ST	27.8 ± 0.20 VW
F35	42.2 ± 0.76 KLM	73.8 ± 0.25 cd	65.0 ± 2.55 mnopqr
F36	43.5 ± 0.76 K	69.0 ± 0.50 gh	65.1 ± 0.55 mnopqr
F37	22.3 ± 0.55 1234	34.3 ± 0.58 ^T	41.7 ± 0.58 KLMN
F38	49.1 ± 0.98 ^I	61.9 ± 1.30 vwxyz	67.3 ± 0.58 hijk
F39	50.0 ± 1.00 ^I	63.7 ± 1.52 qrstuv	64.0 ± 1.00 pqrstu
F40	49.3 ± 1.15 ^I	62.7 ± 0.58 tuvwxy	66.7 ± 1.15 ^{jklm}
	Isolates	Pathogen	Isolate × pathogen
CD ($p \leq 0.05$)	1.46	0.39	2.53
SE(d)	0.74	0.2	1.28
SEM	0.52	0.1	0.9
CV	4.95	4.95	4.95

FOL, *F. oxysporum* f. sp. *lentis*; RS, *R. solani*; BC, *B. cinerea*. Data are the mean of three replicates ± SD; Grouping information among average values was performed by Turkey's HSD method and 95% confidence ($p \leq 0.05$). Different superscript letters and numbers indicate significant differences in a column.

activity against three pathogens (*F. oxysporum* f. sp. *lentis*, *Sclerotium rolfsii*, and *Sclerotinia sclerotiorum*) in lentil (Ramesh and Amit 2019). Plants were constantly exposed to several fungal pathogens in their natural environment, with several diseases often affecting the same crop over its growth cycle (Fernandes *et al.* 2021). However, most biocontrol research focuses on the interactions between a single antagonist and a single pathogen within a particular host-pathogen system (Abdullah *et al.* 2017).

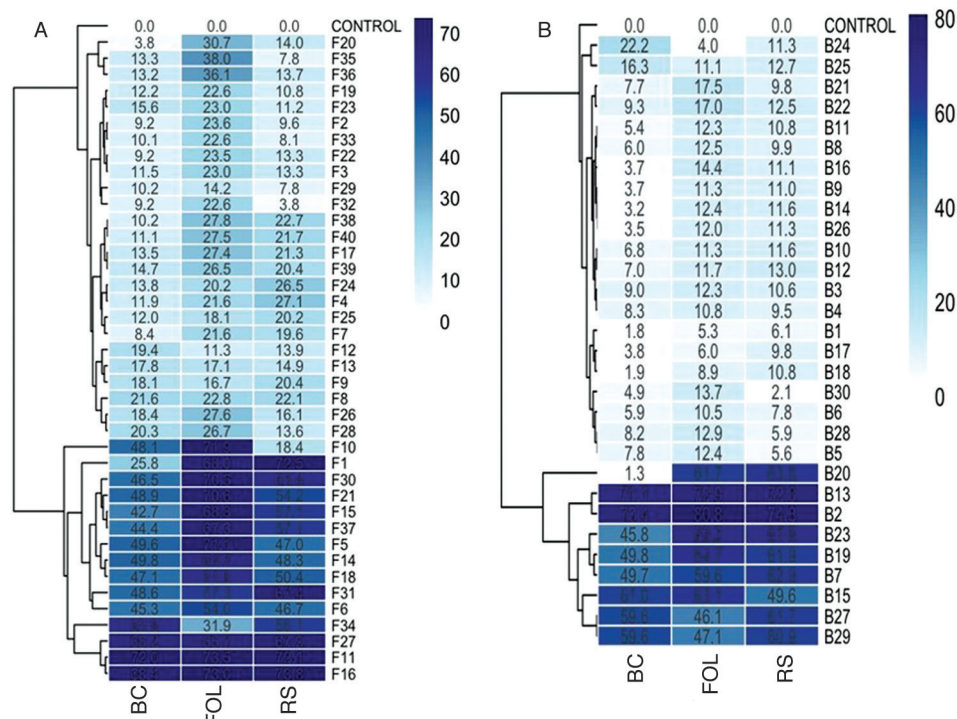


Fig. 2 Heatmap depicting the broad-spectrum. A, Fungal (F1-F40) and B, Bacterial (B1-B30) isolates against *Fusarium oxysporum* f. sp. *lentis* (FOL), *Rhizoctonia solani* (RS), and *Botrytis cinerea* (BC) based on percent inhibition.

Qualitative screening of antagonists for their biocontrol and plant growth-promoting traits: Bacterial and fungal antagonists exhibited diverse plant growth-promoting (PGP) and biocontrol traits, including production of siderophores, hydrogen cyanide (HCN), indole-3-acetic acid (IAA), ammonia, phosphate solubilisation, and hydrolytic enzymes (chitinase, β -1,3-glucanase, cellulase, and chitosanase). However, cellulase activity was absent in all bacterial isolates. Among the tested microbes, fungal isolates F11, F16, and F27 and bacterial isolates B2 and B13 showed the highest overall functional potential and were selected for further studies. The fungal isolates demonstrated strong ammonia (+++) production, efficient phosphate solubilisation, and high activities of chitinase, β -1,3-glucanase, cellulase, and chitosanase. They also produced IAA, siderophores, and HCN, indicating strong rhizosphere competence through nutrient mobilisation and pathogen suppression. Other fungal isolates showed limited or weak trait expression. Similarly, bacterial isolates B2 and B13 revealed the widest range of functional traits, showing strong (++/+++) production of IAA, chitinase, β -1,3-glucanase, chitosanase, ammonia, HCN, siderophore, and phosphate solubilisation. In contrast, other bacterial isolates exhibited partial or weak activity, and the majority lacked siderophore and HCN production (Table 2). These traits enhance plant growth regulation and nutrient accessibility. Siderophore production improves iron uptake, which is necessary for the development of chlorophyll and the activity of the enzymes (Yue *et al.* 2022), while hydrolytic enzymes degrade fungal cell walls, enabling direct antagonism against

R. bataticola (Singh 2017). Auxin production supports root development and stress tolerance (Kaur and Karnwal 2023), whereas microbial HCN production is a critical mechanism for suppressing soil-borne fungal pathogens (Ramette *et al.* 2003).

Morphological and biochemical characterisation of selected broad-spectrum antagonists: A total of 40 fungal and 30 bacterial isolates were screened *in vitro* for plant growth-promoting (PGP) traits and their antifungal efficacy against *F. oxysporum* f. sp. *lentis*, *R. solani*, and *B. cinerea*. Based on their broad-spectrum antagonistic potential (>50% inhibition) and most promising PGP activities, three fungal and two bacterial antagonists were selected for further

characterisation through morphological, biochemical, and molecular identification.

Bacterial isolates B2 and B13 showed distinct traits. B2 formed medium-sized, rod-shaped colonies with uneven margins, flat elevation, opaque appearance, dry coarse texture, and creamy pigmentation. B13 was rod-shaped and produced white to cream-white colonies. Biochemical tests (Gram staining, catalase, indole, KOH, and lipase) were performed. B2 was gram-negative, catalase-positive, lipase-negative, and KOH-positive. B13 was gram-positive, catalase-positive, and negative for both lipase and KOH tests (Table 3). Morphological and microscopic variations observed among the selected fungal isolates (F11, F16 and F27) are presented in Table 3 and Fig. 3.

Morphological traits remain a reliable approach to identify fungal species down to the genus level. The suppression of pathogen growth observed in this study is likely associated with rapid colonisation of the substrate by biocontrol agents, enabling effective competition for space and nutrients prior to the diffusion of extracellular metabolites. Earlier research has shown that catalase-producing bacterial strains are more resilient to mechanical, chemical, and environmental stressors, improve seed emergence, plant growth, and yield, and provide protection against a range of pathogens and pests (Herman *et al.* 2008). In accordance with earlier reports, the morphological characteristics of the selected bacterial isolates resembled those of *Bacillus* spp. isolated from soil, characterised by opaque, flat elevation with irregular margins, and white to greyish-white pigmentation (Kumar *et al.* 2025). These

Table 2 Qualitative screening of fungal and bacterial antagonists for different PGP and biocontrol traits under *in vitro* conditions

Antagonist	Ammonia	P solubilisation	Siderophore	HCN	IAA	Chitinase	Beta-1,3 Glucanase	Cellulase	Chitosanase
F1	+	+	-	-	-	+	+	+	+
F5	+	-	+	-	+	+	+	+	+
F6	-	+	-	-	+	+	+	+	+
F10		-	+	+	+	-	-	+	+
F11	+++	+	+++	+++	++	+++	+++	++	++
F14	-	+	+	-	+	-	+	+	+
F15	-	+	+	-	-	+	+	+	+
F16	+++	+	+++	+++	++	+++	+++	++	++
F18	+	+	-	+	+	+	+	+	+
F21	+	+	+	-	+	+	+	+	+
F27	++	+	++	++	++	+++	+++	++	++
F30	-	+	+	-	-	+	-	+	+
F31	+	+	-	-	+	+	+	+	+
F34	-	+	+	+	+		-	-	-
F37	+	+	+	-	-	+	+	+	+
B2	+++	++	+++	++	+++	+++	+++	-	+++
B7	++	+	++	-	+	-	+	-	-
B13	+++	+++	+++	++	+++	+++	+	-	+++
B15	+	+	-	-	+	+	-	-	-
B19	-	++	-	-	+	++	-	-	++
B20	+	-	++	+	+	-	++	-	-
B23	-	-	-	-	-	++	+	-	++
B27	++	+	-	-	++	++	-	-	-
B29	-	+	+	-	-	+	++	-	-

+++ , High production; ++, Medium production; +, Low production; -, No production.

consistent findings across studies support the multifaceted role of *Bacillus* and other PGPR as nutrient-solubilising and stress-tolerant bioagents, thereby advancing their significance in agricultural sustainability and plant growth promotion.

Molecular identification of broad-spectrum antagonists using 16S rDNA and ITS gene sequencing: The selected isolates were molecularly characterised by amplification and sequencing of the 16S rDNA (bacterial isolates) and ITS regions (fungal isolates), followed by phylogenetic analysis with the NCBI database. Isolate showed 99.50% similarity with *Trichoderma harzianum* isolate ZM24 (PP951515.1), F16 showed 99.19% identity with *T.*

asperellum isolate SISB17 (MH215441.1), and F27 showed 99.65% similarity with *Talaromyces* spp. clone SF717 (MT529993.1) (Fig. 4). Among bacterial isolates, B2 was identified as *Pseudomonas fluorescens* strain ZXCUC (MT712234.1) with 100% similarity, while B13 matched *Bacillus subtilis* strain BSD1 (OL636047.1) with 100% identity. These microbes are widely documented in scientific literature as excellent biocontrol agents and plant growth promoters. *Trichoderma* spp. have antagonistic effects through processes such as mycoparasitism, antibiosis, and the establishment of systemic resistance (Javeria *et al.* 2020), while *Pseudomonas fluorescens* produces antifungal metabolites and enhances plant growth

Table 3 Morphological variation based on qualitative features of the colony among broad-spectrum fungal antagonists

Antagonist	Colour	Concentric rings	Morphology	Pigmentation on the lower side of the plate	Sporulation After (h)	Growth (mm) After 72 h
F11	Dark green	1–2 rings	Circular	Greyish green, central pigmentation	24	82
F16	Dark green	1–2 rings	Circular	Dark green, central dense pigmentation	24	80
F27	Yellow	Absent	Circular	Orange colour	48	71

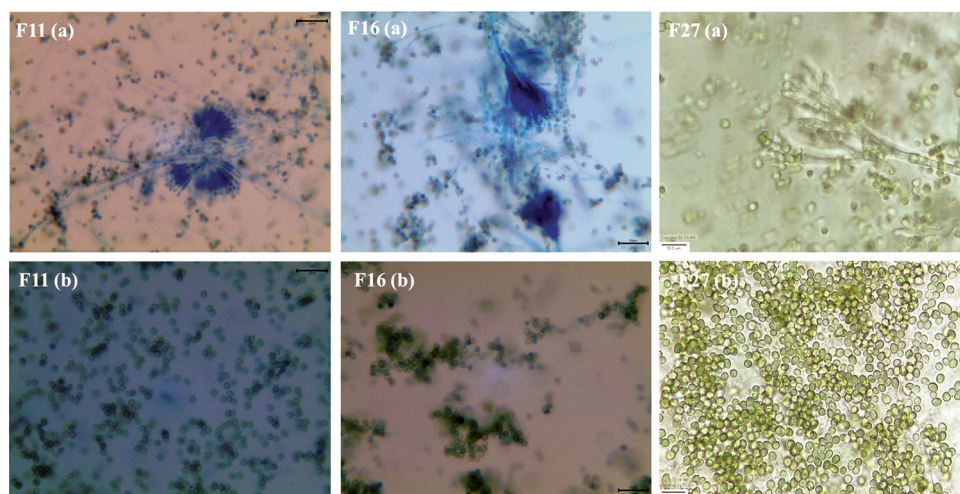


Fig. 3 Microscopic characteristics of broad-spectrum fungal isolates. (a) Phialides (50 µm); (b) Conidia (50 µm).

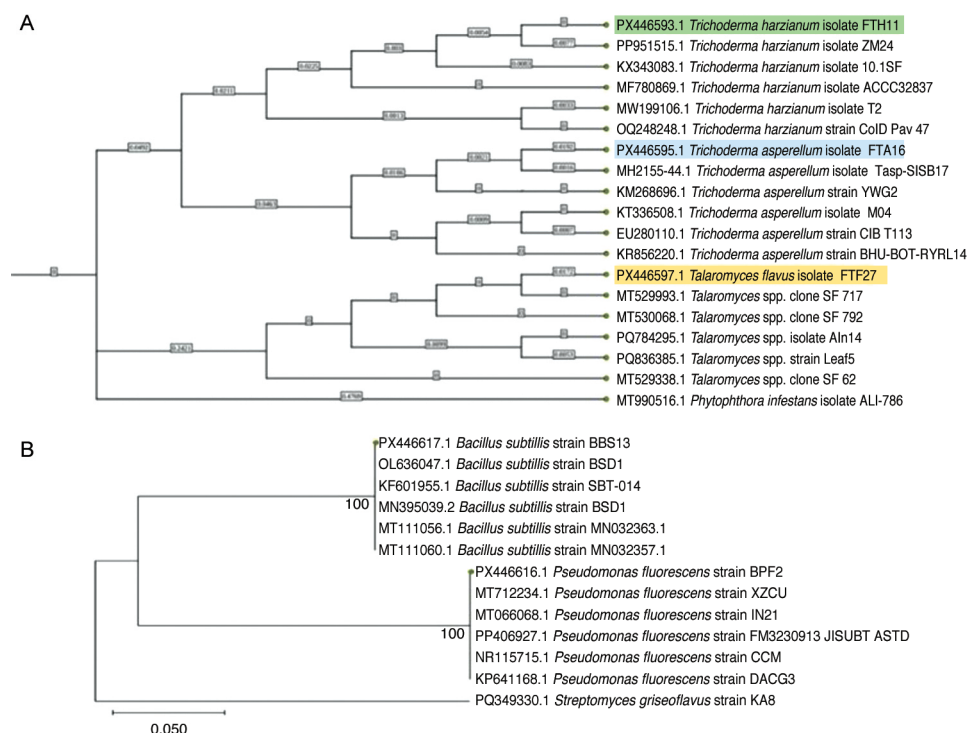


Fig. 4 Phylogenetic relationship among three broad-spectrum fungal (A) and bacterial (B) isolates based on ITS and 16S rDNA sequences by the maximum likelihood method.

(Panpatte *et al.* 2016). The current study emphasizes the significance of these isolates in integrated disease management and sustainable agriculture because their use can minimise dependence on chemical pesticides, lower the risk of pathogen resistance, and provide cost-effective field alternatives. Furthermore, in regions where phosphorus fixation is a major constraint, their high phosphate-solubilising ability improves soil fertility and boosts crop growth. When used together, these bioagents have the potential to significantly increase yields in various cropping systems, while also reducing soil-borne diseases such as wilts and root rots.

The isolation and characterisation of bacterial

and fungal bioagents with broad-spectrum antagonistic activity were the key goals of this study. Using molecular approaches, the isolates were identified as *T. harzianum*, *T. asperellum*, *Talaromyces flavus*, *P. fluorescens*, and *B. subtilis*. These organisms showed significant traits like phosphate solubilisation, IAA production, and antifungal activity, highlighting their dual roles in promoting plant growth and suppressing disease. The findings are encouraging toward large-scale/ commercial utilisation of enhanced antagonistic potential of fungal and bacterial bioagents for environment friendly substitutes for chemical pesticides and effective management of fungal diseases of lentil and hold considerable promise for use in sustainable agriculture.

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