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Screening and characterization of antibiotic producing bacteria from rhizospheric soil against fungal phytopathogens

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ABSTRACT

In this study, antagonistic bacteria were isolated from rhizospheric soil collected from Himachal Pradesh (i.e., Solan and Hamirpur) and Punjab (i.e., Jalandhar and Pathankot). Sixty- eight bacteria were isolated and screened for antifungal activity against *Botrytis oryzae, Aspergillus flavus,* and *Fusarium oxysporum*. Three isolates, i.e., S_7, S_{57}, and S_{51}, showed maximum inhibition for antifungal activity by using the agar streak method and were further selected for *in vitro* characterization. *In vitro* antifungal antibiotic study by using agar diffusion assay of isolates, i.e., S_7, S_{57}, and S_{51} at O.D. 2.0 showed maximum inhibition of 99% against *Botyritis oryzae, Fusarium oxysporum,* and *Aspergillus flavus* after seven days of incubation. Antibiotic assays using cell-free supernatant of antagonistic bacterial isolates, i.e., S_7 and S_{57}, showed complete inhibition at 10% against *Botyritis oryzae* and *Fusarium oxysporum*, whereas isolate S_{51} showed complete inhibition at 20% against *Aspergillus flavus*. The bacterial isolates were partially identified based on morphological and biochemical characteristics. The selected antagonistic bacterial isolates S_7 and S_{57} appeared to be similar to *Bacillus sp*. and S_{51} to *Actinomycete sp*. Thus, the present study concludes that these bacterial isolates could serve as proficient biocontrol inoculants in the integrated management of soil-borne diseases in different crops under natural environmental conditions.

KEYWORDS

Antibiotics; Rhizosphere; Antifungal activity; *Actinomycetes* sp.; *Bacillus* sp.

ARTICLE HISTORY

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Introduction

Since the widespread use of chemicals has a detrimental effect on the environment and human health, biological control is a desirable alternative to chemical pesticides for protecting plants from infections. By testing the huge number of soil or plant-associated microorganisms for antagonistic activity against phytopathogens *in vitro* or in planta, several biocontrol agents were identified. Microorganisms have developed several ways to inhibit their neighbors by producing certain types of substances [1,2]. These substances are secondary metabolites, which are produced by one microorganism to inhibit the growth of another microorganism, for example, antibiotics [3]. Soil is the main source of antibiotic-producing microorganisms [4]. An antibiotic made by a soil microbe has the ability to inhibit any other soil microbes [5]. Soil contains many bacterial genera along with many species of fungi. Bacterial species like *Bacillus, Streptomyces,* and *Actinomycetes* are present in soil. Fungal species like *Fusarium, Botrytis, Aspergillus, Penicillium,* and *Cephalosporium* are residents and cause various diseases in plants [2,6]. Only a few antibiotics have been used commercially to treat human and plant diseases from the cultivation of gram-positive, gram-negative, and filamentous fungi. [7,8]. Although it is widely acknowledged that the soil is full of bacteria that may synthesize antibiotics, it is less well understood how frequently synthesis occurs in nature at ecologically important levels. Gram-positive bacteria, classified as actinomycetes, are a class of branching unicellular microorganisms that are well known for their capacity to manufacture antibiotics [9]. Streptomycetes are the most prevalent actinomycetes. *Bacillus* sp. is a significant species of bacterium that can manufacture antibiotics. According to Ball and Bartlett, the genus contains gram-positive, endospore-forming, chemoheterotrophic rods that are typically motile and have peritrichous flagella [10]. Gramicidin, a linear polypeptide antibiotic combination of gramicidin A, B, C, and D, is produced by the bacterium *Bacillus brevis*. The largest genus, Streptomyces, has about 150 species that are strictly aerobes, have cell walls of type I, and form chains of non-motile Streptomyces species are identified using a combination of morphological and physiological traits and are involved in the manufacture of antibiotics. The issue of bacteria developing resistance to the current antibiotics gets worse every day. As a result, finding novel antibiotics or the sources of new antibiotics is urgently needed [11-13].

By engaging in antagonistic interactions with plant diseases, soil bacteria in the rhizosphere can promote plant growth. In this work, rhizospheric antagonistic bacterial isolates will be isolated and characterized for their *in vitro* antifungal activity against the fungal diseases *Botyritis oryzae, Fusarium oxysporum,* and *Aspergillus flavus*. These fungi cause several fungal diseases in plants, including complicated root rot and bacterial wilt.

Materials and Methods

Sampling of soil

Soil samples were collected from rhizosphere soil from different locations in Punjab (Jalandhar and Pathankot) and Himachal Pradesh (Hamirpur and Solan). Representative samples of soil were collected randomly from 0-15cm and 15-30cm depths of the rhizosphere. At least three subsamples of soil were pooled to form a composite sample. All soil

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samples were stored in clean polythene bags and brought to the laboratory. The soil samples were air-dried, ground, and passed through a 2mm sieve for physicochemical analysis. Soil samples were stored in a humid atmosphere (80% relative humidity) at 20 °C for a maximum of seven days.

Physical and chemical analysis of the soil

Freshly procured samples of the soil were analyzed first for their physical and chemical characteristics. The soil pH and conductivity were determined in a 1:5 soil: water suspension. The mixture was shaken for 2 hours, and the supernatant was filtered and used for measurement. Soil organic carbon content was determined by the hydrometer method [14] and the Walkley Black wet oxidation method [15], respectively. A factor of 1.72 was multiplied by organic carbon content to determine soil organic matter (SOM). Potassium (K) was measured using a Systronics flame photometer after digesting the samples in a diacid mixture $HClO_4/HNO_3$ in a 4:1 ratio) [16]. Total nitrogen (N) was determined by the Kjeldahl method [17], and available phosphorous (P) by the sodium bicarbonate extraction method using Systronics spectrophotometer [18].

Isolation and enumeration of bacteria

Bacteria were isolated from rhizosphere soils collected from different locations in Himachal Pradesh (Solan, Hamirpur) and Punjab (Jalandhar) during the months of February and March. Soil and roots were kept in plastic bags and stored at 0 °C for a maximum of 7 days for processing. The serial dilution plating method was used for the isolation of bacteria from soil, and an aliquot of 0.1-1ml of diluted sample was plated onto a complex non-selective nutrient agar medium and spread using an L-shaped spreader. The plates were then incubated at 37 °C for 3 days. Isolates were restreaked on NAM (pH 7.0) of the following composition (g/l): peptone, 5.0; beef extract, 3.0; NaCl, 5.0; agar, 20.0; checked for purity; and stored on NAM slants at 4 °C. The bacterial culture was maintained in 30% glycerol at -20 °C.

In vitro testing of the bacterial antagonists by agar streak method

A loopful of 48-hour-old culture of each isolate was streaked individually below the center of the pre-poured potato dextrose agar (PDA) Petri plates to test the effectiveness of the bacterial antagonists. A mycelial disc of the test fungal pathogen was applied at the center of the Petri plate. For comparison, control without bacterial streak and only inoculated with the test pathogen was used. Three replications of each treatment were performed. According to Vincent, the percentage of growth inhibition was estimated after the plates were incubated at 24 °C for 7 days [19].

$$I = \frac{C - T}{C} \times 100$$

Where,
I= Per cent growth inhibition
C= Growth of fungus in control
T= Growth of fungus in treatment

Antibiotic assay by agar diffusion assay

Before plating of PDA, bacterial cell suspension (2ml) with various population densities (OD 0.5, 1.0, 1.5, and 2.0 at 540 nm) was added to study the inhibition of the fungus. The fungal bit was positioned in the center of the plates and left to grow for 24 ± 1 °C. In the control, the bacterial cell suspension was replaced merely with nutritional broth. Three replications of each treatment were performed. After 7 days of incubation, the amount of fungal growth inhibition was measured and estimated according to Vincent [19].

Antibiotic assay by cell-free supernatant technique

With just minor changes, the Nora and Bettache method was utilized to assess the activity of bacterial cell-free supernatants [20]. After 24 hr of incubation at 30 °C of bacteria in nutritional broth, the bacterial cultures were centrifuged at 10,000-12,000 rpm for 10-15 minutes, and the resultant supernatant was filtered through a Millipore filter of size, 0.2 µm. 5 to 20% of cell-free supernatant was spread on a petri plate containing potato dextrose agar. In the case of 5% cell-free supernatant, 1ml of cell-free supernatant and 19ml of PDA medium were used. The center of the plates was inoculated with a freshly grown fungal bit on a PDA medium. Fungus-filled control plates lacking cell-free supernatant were developed. For seven days, fungal growth on the petri plates was monitored after every 24 hr of incubation at 24 ± 2 °C. Examining and comparing the inhibition zones with the control.

Morphological and biochemical characterization

Gram's reaction, colony morphology, and cell shape were examined for the morphological characterization of selected bacterial antagonists. Selected isolates were also characterized biochemically for H_2S production, fermentation test, as well as tests for catalase, indole production, methyl red, Voges-Proskauer, cellulase test, and amylase test.

Results

The physico-chemical properties of soils are given in Table 1. The studied soil samples were found to be slightly acidic to neutral. The soil sample collected from Solan showed an acidic nature, whereas all the other soil samples were neutral in nature. The pH of soils ranged from 6.42-7.4. Soil electrical conductivity, which is a major indicator of the salinity of soil, was found to be low (0.17-0.21 dSm^{-1}). Soil organic matter (SOM) content ranges from 2.79-3.23% and was found to be highest in a soil sample collected from Jalandhar. Various soil nutrients such as nitrogen (N), phosphorus (P), and potassium (K) ranged from 225-255kg/ha, 16-20kg/ha, and 185-215kg/ha, respectively.

Table 1. Physico-chemical characterization of soil collected from different locations.

Soil sample location	pH	SOM (%)	N (kg/ha)	P (kg/ha)	K (kg/ha)	EC (dSm^{-1})
Solan	6.42	2.98	225	19	200	0.17
Hamirpur	7.0	2.79	230	16	185	0.18
Jalandhar	7.4	3.12	255	20	210	0.22
Pathankot	7.2	3.23	245	18	215	0.21

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Isolation and enumeration of bacterial isolates for antifungal activity

To check the antifungal properties, different bacteria were isolated from the soil (Figure 1). The soil was collected from various locations, i.e., Solan, Hamirpur district of Himachal Pradesh, and Pathankot, Jalandhar district of Punjab, based on different soil properties, during the months of February and March. The bacterial population on the nutrient agar plate was enumerated and reported as CFU/g of soil. A total of 68 bacteria that are morphologically different from each other were isolated from distinct locations of soil. These 68 bacterial isolates were checked for their antifungal properties against *Botrytis oryzae, Fusarium oxysporum,* and *Aspergillus flavus*. Among samples of four distinct locations, sample 4, isolated from Pathankot, had the highest population density (54 × 10^5cfu/g of soil), and sample 2, isolated from Hamirpur, had the minimum population density (24 × 10^5cfu/g of soil), as shown in Table 2. The difference in number may be due to the different physical characteristics of the soil present at various places.

Table 2. Enumeration of bacterial isolates for antifungal activity isolated from rhizospheric soil of different locations of Punjab and Himachal Pradesh.

Samples	Location	Bacterial population on nutrient agar medium isolated from soil of different locations (× 10^5cfu/g of soil)
Sample 1	Solan	49 ± 3.0
Sample 2	Hamirpur	24 ± 2.0
Sample 3	Jalandhar	32 ± 4.0
Sample 4	Pathankot	54 ± 5.0

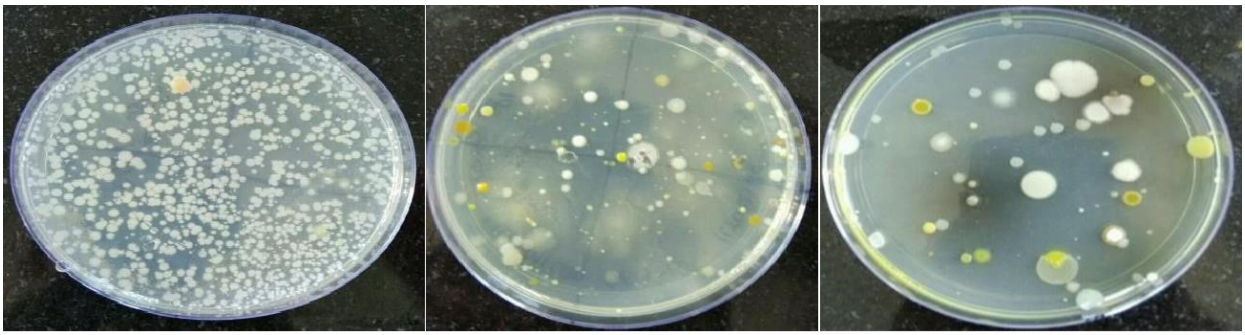

Figure 1. Isolation of bacteria from rhizospheric soil on nutrient agar medium at different dilution rates.

Screening of different bacterial isolates

The isolated bacteria were screened against the three different fungal pathogens, i.e., *Botyritis oryzae, Fusarium oxysporum,* and *Aspergillus flavus,* on Potato Dextrose Agar (Figure 2). Out of 68 isolates that were tested for their antifungal action against three different fungal pathogens, only four, i.e., S_1, S_2, S_6, and S_7, showed positive results against *Botyritis oryzae*, isolate S_{57} was positive against *Fusarium oxysporum*, and isolates S_{51} and S_{65} were positive against *Aspergillus flavus*. Some isolates showed contact inhibition against three fungal pathogens, but they were not selected for further study.

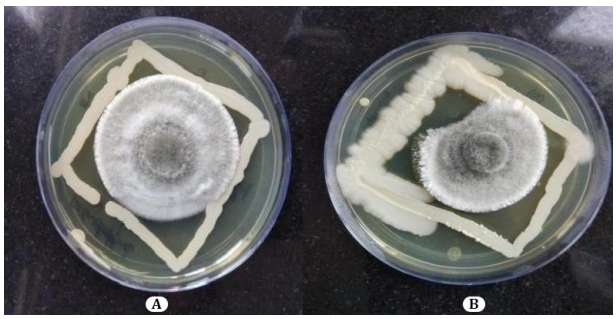

Figure 2. Screening of S_2, S_6 and S_7 bacterial isolates for antifungal activity against *B. oryzae* on PDA medium.

Percent growth inhibition of antagonistic bacteria by agar streak method

Bacterial isolates, i.e., S_1, S_2, S_6, and S_7, were capable of inhibiting the fungal pathogen, i.e., *B. oryzae*. Maximum growth inhibition was found for isolates S_6 (48%) and S_7 (48%) as compared to S1 and S2 against *B. oryzae*, as shown in Table 3. Bacterial isolates, i.e., S_{48} and S_{57}, were capable of inhibiting the fungal pathogen, i.e., *F. oxysporum*. Out of which, it was found that S_{48} (27.5%) showed contact inhibition, and S_{57} (47.5%) was capable of inhibiting the growth of *F. oxysporum*, as shown in Table 3. Bacterial isolates, i.e., S_{19}, S_{51}, and S_{65}, were capable of inhibiting fungal pathogen, i.e., *A. flavus*. Out of which, it was found that S_{19} (25%) showed contact inhibition and S_{51} (47.5%) was capable of inhibiting the growth of *A. flavus*, as shown in Table 3.

Effect of cell density of selected antagonistic bacterial isolates on fungal pathogens

The findings of the effect of cell density of S_7 isolate on the growth of *B. oryzae* showed that the percent growth inhibition of the fungus was found to be about the same in all the bacterial cell densities, i.e., 0.5, 1, 1.5, and 2, ranging from 97.5

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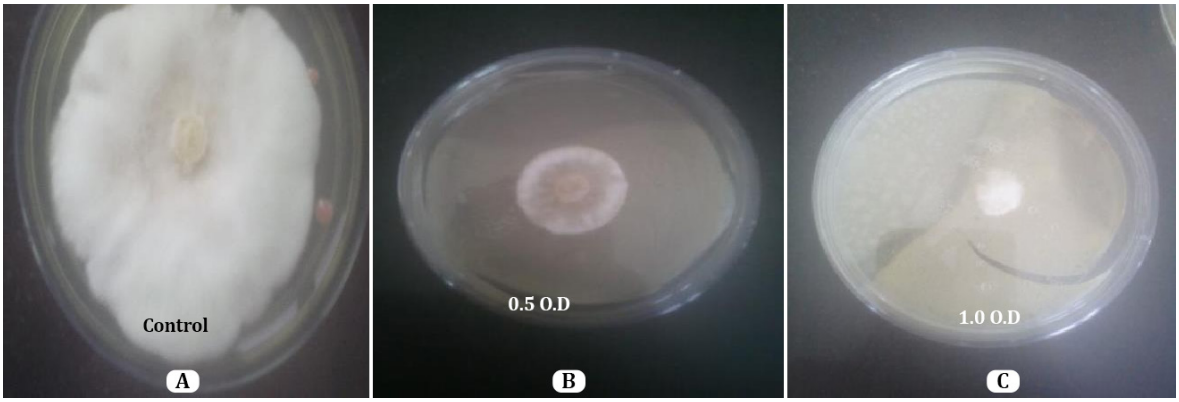
Table 3. Percent growth inhibition of antagonistic bacterial isolates against *Botrytis oryzae, Fusarium oxysporum,* and *Aspergillus flavus* on PDA medium.

Fungal Pathogen	Isolates	Control (C) (cm)	Treatment (T) (cm)	% Growth inhibition (I*)
	S_1	3.5	2.2 ± 0.03	37.14
Botyritis oryzae	S_2	3.5	2.6 ± 0.05	25.70
	S_6	3.5	1.9 ± 0.02	45.71
	S_7	3.5	1.8 ± 0.03	48.57
Fusarium	S_{48}	4.0	2.9 ± 0.02	27.50
oxysporum	S_{57}	4.0	2.1 ± 0.01	47.50
Aspergillus flavus	S_{19}	4.0	3 ± 0.02	25.00
	S_{51}	4.0	2.1 ± 0.05	47.50
	S_{65}	4.0	2.5 ± 0.06	37.50

to 99%, which was at par with each other. In 0.5 O.D., the growth inhibition was 97.5%; in 1 O.D., the growth inhibition was 98%; and in 1.5 and 2 O.D., the growth inhibition was 98.5 and 99%, as shown in Table 3. The effect of isolate S_{57} on the growth of *F. oxysporum* showed that the percent growth inhibition of the fungus was found to be the same in all the bacterial cell densities, i.e., 0.5, 1, 1.5, and 2, ranging from 97.5 to 99%, which was at par with each other. In 0.5 O.D., the growth inhibition was 97.5%; in 1 O.D., the growth inhibition was 98.5%; and in 1.5 and 2 O.D., the growth inhibition was 99%, as shown in Table 3 and Figure 3. The effect of the cell density of isolate S_{51} on the growth of *A. flavus* showed that the percent growth inhibition of the fungus was found to be the same in all the bacterial cell densities, i.e., 0.5, 1, 1.5, and 2, ranging from 97.5 to 99%, which was at par with respect to each other. In 0.5 O.D., the growth inhibition was 97.5%; in 1 O.D., the growth inhibition was 98%; and in 1.5 and 2 O.D., the growth

Table 4. Effect of cell density of selected antagonistic bacterial isolates on the growth of *Botrytis oryzae, Fusarium oxysporum,* and *Aspergillus flavus*.

Isolate	Cell density (O.D)	Growth of *Botrytis oryzae* (cm)	% growth inhibition (I)
S_7	0.5 ± 0.01	0.1	97.50 %
	1.0 ± 0.02	0.06	98.00 %
	1.5 ± 0.01	0.05	98.50 %
	2.0 ± 0.03	0.04	99.00 %
	Cell density (O.D)	Growth of *Fusarium oxysporum* (cm)	% growth inhibition (I)
	0.5 ± 0.01	0.1	97.50 %
	1.0 ± 0.01	0.06	98.50 %
S_{57}	1.5 ± 0.06	0.05	99.00 %
	2.0 ± 0.02	0.04	99.00 %
	Cell density (O.D)	Growth of *Aspergillus flavus* (cm)	% Growth inhibition (I*)
	0.5 ± 0.03	0.1	97.50 %
	1.0 ± 0.03	0.08	98.00 %
S_{51}	1.5 ± 0.05	0.05	99.00 %
	2.0 ± 0.01	0.05	99.00 %



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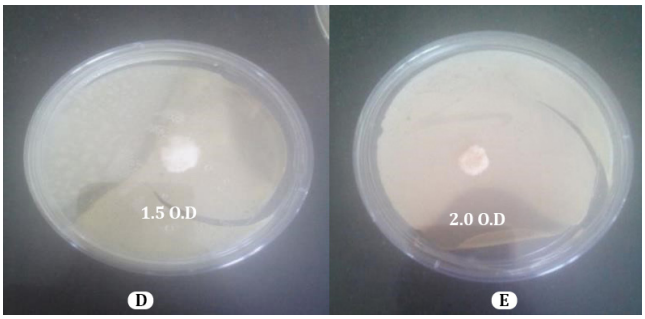

Figure 3. Effect of cell density of antagonistic bacterial isolate (S_{57}) on the growth of *Fusarium oxysporum*.

inhibition was 99%, as shown in Table 4.

Antibiotic assay of antagonistic bacterial isolates by cell-free supernatant technique against fungal pathogens

The effect of the antibiotic assay of antagonistic bacterial isolates by cell-free supernatant technique against *B. oryzae* revealed that the concentrations of cell-free supernatant of isolate S_7 used in this experiment completely inhibited the growth of fungus in the antibiotic assay technique. Different concentrations, i.e., 10% and 20%, were used, and the 10% concentration was found to be completely inhibiting the growth of the fungus. The rest of the concentrations similarly inhibited the growth of fungus and were at par with each other, as shown in Figure 4. The results of the antibiotic assay of antagonistic bacterial isolates by the cell-free supernatant technique against *F. oxysporum* showed that the concentrations used in this experiment completely inhibited the growth of fungus in the antibiotic assay technique. Different concentrations of cell-free supernatant of isolate S_{57}, i.e., 10% and 20%, were used, and both concentrations completely inhibited the growth of the fungus, i.e., *F. oxysporum*. The effect of the antibiotic assay by cell-free supernatant technique against *A. flavus* revealed that the 10% and 20% concentrations of isolate S_{51} were used to inhibit the fungus. Maximum inhibition was found at a concentration of 20% that completely inhibited the growth of *A. flavus*, whereas 95% growth inhibition was found when a concentration of 10%

Figure 4. Antibiotic assay using antagonistic bacterial isolate (S_7) by cell-free supernatant technique against *A. flavus*.

of the cell-free supernatant of isolate S_{51} was used.

Morphological and physiological characterization of selected antagonistic soil bacterial isolates

Numerous bacterial species were isolated from rhizospheric soil, and their cellular and colonial morphologies were characterized. The morphological properties, such as shape, color, and type of colony formation, as well as physiological and biochemical traits, were used to characterize and identify isolates. The isolated colonies on nutrient agar medium were circular in form, had a smooth surface, flat elevation, and an undulating edge, and were colored yellow and cream. The morphological properties of the isolates were examined and found to be rod-shaped, moderately sized, and organized singly and in chains (Table 5).

Table 5. Morphological characteristics of selected antagonistic bacterial isolates S_7, S_{57} and S_{51}.

Isolates	Colony Characteristics				Gram Reaction
	Colour	Configuration	Margin	Elevation	
S_7	Cream	Round	Smooth	Flat	+ve
S_{57}	Cream	Filamentous	Smooth	Flat	+ve
S_{51}	Cream	Branching	Colony	Flat	+ve

Biochemical characterization of selected antagonistic soil bacterial isolates

Antagonistic bacterial isolates, i.e., S_7, S_{57}, and S_{51}, were tested for H_2S production, citrate utilization, indole production, amylase test, cellulose test, methyl red test, and Vogus-Proskauer test. All biochemical tests were positive except the Vogus-Proskauer and amylase tests. Based on morphological and biochemical characterization, the selected

Table 6. Biochemical characterization of selected antagonistic bacterial isolates S_7, S_{57} and S_{51}.

Biochemical Test	S_7	S_{57}	S_{51}
H_2S production	+	+	+
Fermentation test	+	+	+
Amylase test	-	-	-
Methyl red test	+	+	+
VP Reagent test	-	-	-
Catalase test	+	+	+
Cellulase test	+	+	+

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bacterial antagonists, i.e., S_7 and S_{57}, were found to be *Bacillus* sp., and the bacterial isolate S_{51} was *Actinomycete* sp. (Table 6).

Discussion

Soil is a habitat for numerous types of microorganisms and has a wide range of uses in agriculture, pharmacy, and medicine [21,22]. Soil properties are the main factors affecting the distribution and composition of soil microorganisms [23]. The physico-chemical properties of soils are given in Table 1. The studied soil samples were found to be slightly acidic to neutral in nature. Microorganisms are the most active component of the soil and are found more in neutral soil. The microbial community structure and function respond very rapidly to changes in the microenvironment. The soil organic matter (SOM), which is the storehouse of plant nutrients and mineral recycling [24], was found to be low, which can be attributed to the sandy texture of the soil. Various soil nutrients, such as nitrogen (N), phosphorus (P), and potassium (K), were analyzed. All these soil parameters analyzed have a significant effect on the number of microorganisms present in the rhizosphere. Microbial communities are an important biological indicator of the health status of soil and maintaining soil fertility [25].

Screening of antifungal bacterial isolates of soil was done by using the agar streak method on potato dextrose agar (PDA) medium against *Botyritis oryzae, Fusarium oxysporum,* and *Aspergillus flavus* [26,27]. Out of sixty-eight isolates, six isolates, viz., S_6, S_7, S_{57}, S_{51}, and S_{65}, were selected. Three isolates, i.e., S_7, S_{57}, and S_{51}, were further selected for screening and characterization based on maximum percent growth inhibition against *Botyritis oryzae, Fusarium oxysporum,* and *Aspergillus flavus*.

In the previous study conducted by Prashar et al., the percent growth inhibition of isolate TNAM5 was found to be 47.77%, which is nearly similar to the present study for isolates S_6 and S_7, i.e., 48% [28]. Another study conducted by Kaur showed that isolate N9 showed maximum percent growth inhibition, i.e., 60.61% against *B. oryzae*, and minimum growth inhibition of fungi was shown by isolate B2, i.e., 39.39% against *B. oryzae*, which was also in agreement with the present findings [29]. Yuan et al. found that *Bacillus subtilis* E11 screened from 63 candidate antagonistic bacteria exhibited the strongest antifungal inhibition (64.27%) against *A. flavus* [30].

In a study conducted by Walia et al., bacteria isolated from the tomato rhizosphere showed maximum percent growth inhibition against *F. oxysporum* (42.57%), *R. solani* (47.30%), and *S. sclerotiorum* (487.1%), which was in agreement with the present findings [31]. Kaur showed related results where isolate N9 showed the maximum percent growth inhibition, i.e., 52.63%, against *F. oxysporum*, which was similar to the present findings [29]. An *in vitro* assay was performed by Balthazar et al. to see the percentage of fungal growth inhibition achieved by the 12 *Bacillus sp.* and *Pseudomonas sp*. and calculated the reduction of mycelium diameter in dual culture petri plates relative to control plates [32]. Finally, the three most significant inhibitory bacteria were *Bacillus velezensis* LBUM279, FZB42, and *Pseudomonas synxantha* LBUM223 against *Botrytis cinerea*, with inhibition rates ranging from 50 to 70%, closely followed by *B. velezensis* LBUM1082, *B. subtilis* LBUM979, and *P. protegens* Pf-5 with inhibition rates >40%.

According to Barnossi et al. results, isolate Gn-A11-18 showed significant inhibitory effects with a 42.66% inhibition percentage against *A. niger* and a 44.66mm inhibition diameter [2]. These findings were similar to the findings from the current study, where isolate S_{51} demonstrated a 47.5% growth inhibition against *A. flavus* when compared to the control.

Bacterial cell density showed a maximum inhibition of 99% against all three fungal pathogens. In a previous study conducted by Walia et al., the percent growth inhibition of the fungus increased significantly from 49.94 to 58.53% with an increase in bacterial cell density, in contrast to our results, which showed maximum inhibition of 99% at OD 2.0 [31]. This suggests that the maximum concentration of bacteria had maximum inhibitory properties and was capable of inhibiting the growth of fungus against all three fungal pathogens. These results are in agreement with an earlier report that *Bacillus sp.* produced antifungal substances with activity against a number of mycelia fungi [33,34]. We suppose that this effect is caused by different antifungal metabolites, including siderophores, organic acids, IAA, and antifungal antibiotics in the cell pellets [35].

Antibiotic assay of antagonistic bacterial isolate by cell-free supernatant technique showed complete inhibition of the growth of fungus against all three fungal pathogens in antibiotic assay technique. Different concentrations of cell-free supernatant of the isolate, i.e., 10% and 20%, were used, and both concentrations completely inhibited the growth of all three fungi. These results agree with the findings given by Kim and Kang, where cell-free supernatants obtained from the probiotic *Pediococcus acidilactici* HW01 showed antifungal activities against *Candida albicans* [1].

The fungicidal actions of the PGPR's metabolites on fungal infections may have caused their antagonistic effects. According to Gamliel and Katan, *Pseudomonas fluorescens* isolates that produce siderophores and chelate iron are antagonistic to *F. oxysporum* [36]. *Bacillus megaterium, Bacillus subtilis,* and *Pseudomoas fluorescens* were also shown to decrease the population of *F. oxysporum* in the rhizosphere in previous research [37,38].

Conclusions

Isolates S_7, S_{57}, and S_{51} showed maximum antifungal activity against all three fungal pathogens. *In vitro* antifungal antibiotic studies showed all three isolates showed maximum inhibition of 99% against *Botyritis oryzae, Fusarium oxysporum,* and *Aspergillus flavus* after seven days of incubation at O.D. 2. Antibiotic assays using cell-free supernatant of antagonistic bacterial isolates S_7 and S_{57} showed complete inhibition at 10% against *Botyritis oryzae* and *Fusarium oxysporum*, whereas isolate S_{51} showed complete inhibition at 20% against *Aspergillus flavus*. These antifungal properties of bacteria can be useful to plants for generating resistance against different types of fungal pathogens and are an effective method to substitute chemical fungicides for sustainable crop cultivation. An efficiency test under field conditions needs to be conducted to see the role of these antifungal bacteria against the pathogenic effects of fungal pathogens for disease-free plant growth and development. Molecular characterization of the strains will be required to find out the species of the strain. Further studies of genes showing antimicrobial activities in bacteria may be helpful in providing resistance to plants

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against various fungal infections by generating genetically modified plants.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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