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Research Article

Biocontrol potential of endophytic actinomycetes from *Azadirachta indica* and *Datura innoxia*: Assessment of antifungal activity against phytopathogens

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ABSTRACT: Endophytes cause unapparent and asymptomatic infections and live entirely within plant tissues. Endophytes improve growth of host plants and protect from biotic and abiotic stresses. The present study was conducted to evaluate the antagonistic activity of endophytic filamentous actinobacteria isolated from *Azadirachta indica* and *Datura innoxia* against fungal phytopathogens, *viz*., *Alernaria alternata, Alernaria brassicicola, Alernaria mali, Alernaria tenuissima, Colletotrichum capsici, Colletotrichum gloeosporioides, Fusarium moniliforme, Fusarium oxysporum, Fusarium sacchari, Ustilaginoideae virens, Ustilago tritici.* A total of 10 endophytic actinomycetes were isolated. The maximum number of endophytic filamentous actinomycetes was isolated from leaves. Leaves yielded 5 out of 10, *i.e.*, 50% of endophytic filamentous actinobacteria. One filamentous actinobacterial isolate, DSA-1 (morphological resemblance to *Streptomyces* sp.), showed significant and broad-spectrum activity against fungal phytopathogens. Filamentous actinobacterial isolate DSA-1 showed significant activity against *A. alternata, C. capsica, F. moniliforme, F. sacchari, Ustilaginoidae virens, Ustilago tritici* and no activity against *A. brassicicola*, *A. mali*, *A. tenuissima, C. gloeosporioides* and *F. oxysporum*. Maximum antagonistic activity was observed against *U. virens* (22.8 mm). Thus, the filamentous actinobacterial endophytic isolate showed antagonistic activity against fungal phytopathogens and may be used as a biological control agent.

KEYWORDS: Antagonistic activity, *Azadirachta indica*, *Datura innoxia*, endophytic actinobacteria, fungal phytopathogens

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INTRODUCTION

Agricultural production worldwide is continuously challenged by plant diseases caused by a wide range of phytopathogenic microorganisms, among which fungal pathogens are particularly important because of their ability to infect diverse crops and cause substantial reductions in yield and quality. Fungal diseases affect cereals, pulses, oilseeds, vegetables, fruits, and other economically important crops, resulting in losses at both pre- and post-harvest stages (Fisher *et al*., 2012; Pandey *et al*., 2021; Cucu *et al*., 2025). The management of fungal plant diseases has traditionally relied heavily on synthetic fungicides. Although chemical pesticides can provide rapid disease suppression, their extensive and repeated application may result in environmental contamination and undesirable effects on non-target organisms (Aktar *et al*., 2009). Accumulation of pesticide residues and concerns about their potential effects on human and animal health have further encouraged the development of alternative approaches to crop protection (Aktar *et al*., 2009).

Endophytes are microorganisms that colonise the internal tissues of living plants without producing apparent disease symptoms or visible detrimental effects under the conditions in which the association is observed (Golinska *et al*., 2015; Khare *et al*., 2018). They may occur within

roots, stems, leaves, flowers, fruits, seeds, and other plant organs, and their distribution can vary according to plant tissue and environmental conditions. The plant endosphere therefore represents a distinct microbial habitat in which microorganisms interact closely with host tissues and may influence plant growth, nutrient acquisition, stress tolerance, and interactions with other microorganisms (Khare *et al*., 2018). Endophytic actinobacteria constitute an especially promising group for the discovery of microorganisms with agricultural and pharmaceutical applications. Actinobacteria are predominantly Gram-positive microorganisms characterised by diverse morphological and physiological properties, with many members possessing relatively high genomic G+C contents (Barka *et al.*, 2016). Members of the phylum are particularly recognised for their extensive secondary metabolism and their ability to synthesise structurally diverse natural products (Barka *et al*., 2016; Passari *et al*., 2017). The genus *Streptomyces*, in particular, represents one of the most important sources of naturally occurring antimicrobial compounds and other bioactive metabolites (Golinska *et al*., 2015). Endophytic actinobacteria isolated from medicinal plants have been reported to exhibit antimicrobial, plant-growth-promoting, enzyme-producing, and other functional properties of potential agricultural value (Golinska *et al*., 2015; Passari *et al*., 2015). Their occurrence within plant tissues also provides an opportunity to explore microbial populations inhabiting ecological niches that may differ considerably from conventional soil environments (Aamir *et al.*, 2020).

The search for endophytic actinobacteria is also relevant to agricultural environments in which locally adapted microbial populations may possess characteristics influenced by their host plants and surrounding environmental conditions. Endophytic actinobacteria associated with crop plants have been reported to exhibit antimicrobial and plant-growth-promoting activities, demonstrating the functional diversity present within plant-associated microbial communities (Shan *et al*., 2018; Borah & Thakur, 2020). The isolation of actinobacteria from plants growing under particular environmental conditions may consequently provide access to microbial populations adapted to specific ecological settings (Jiang *et al*., 2018). Such locally associated microorganisms may represent useful resources for subsequent evaluation as biological control candidates. Nevertheless, the functional potential of an isolate cannot be inferred solely from its plant origin and requires experimental assessment against relevant phytopathogens.

Despite the considerable potential of endophytic actinobacteria, the biological activity of isolates obtained from locally associated medicinal plants remains insufficiently explored in many agro-ecological regions. Previous investigations have demonstrated that endophytic actinomycetes isolated from medicinal plants can possess antagonistic activity against important phytopathogenic fungi (Passari *et al*., 2015; Singh *et al.,* 2017). Similarly, endophytic actinobacteria recovered from other plant hosts have demonstrated antimicrobial activity and diverse plant-beneficial traits (Zhao *et al*., 2011). However, the spectrum of antagonistic activity can differ substantially among isolates and target pathogens, emphasising the importance of screening individual isolates against a diverse collection of phytopathogens. Comparative evaluation of isolates obtained from different tissues of *A. indica* and *D. innoxia* may therefore provide useful information for identifying candidates with relatively broad antifungal activity.

Therefore, the present study was undertaken to isolate and purify endophytic actinobacteria from different tissues of *Azadirachta indica* and *Datura innoxia* and to evaluate their antagonistic activity against agriculturally important fungal phytopathogens. Endophytic actinobacterial isolates obtained from leaves, stems, roots, flowers, fruits, and bark were screened for antagonistic activity, initially using *Colletotrichum capsici*, followed by evaluation of active isolates against a broader range of fungal pathogens belonging to *Alternaria*, *Colletotrichum*, and *Fusarium*, as well as the cereal pathogens *Ustilago tritici* and *Ustilaginoidea virens*. The study was designed to identify endophytic actinobacterial isolates exhibiting antifungal activity against multiple phytopathogens and to assess their potential as microbial resources for further investigation in biological disease management.

MATERIALS AND METHODS

Collection of plant parts

Plant material for endophyte isolation was collected from visibly healthy, mature, and disease-free specimens of *Azadirachta indica* and *Datura innoxia*; two medicinal plants selected based on their documented therapeutic properties, traditional household use, and local availability. The sampling included various plant parts—leaves, flowers,

fruits, stems, bark, and roots—to ensure a comprehensive representation of endophytic diversity. Collections were made from both the campus and adjacent field areas of Guru Kashi University, located in Bathinda, Punjab, India. This region lies in the northwestern part of the Indian subcontinent within the fertile Indo-Gangetic plains.

Isolation of endophytic actinobacteria

The samples were first washed under running tap water to remove adhered debris and soil particles and then washed with distilled water. For isolation of Endophytic actinobacteria, plant tissues (roots, stems and leaves) were sterilised by sequential immersion in 70% ethanol for 5 min and sodium hypochlorite solution (0.9% w/v, available chlorine) for 20 minutes, and then surface-sterilised samples were washed in sterile water three times to remove surface sterilisation agents. Then the samples were soaked in 10% (w/v) sodium bicarbonate solution to retard the growth of endophytic fungi. Each tissue was divided into small fragments (0.5 cm × 0.2 cm) and was placed on isolation medium, Starch Casein Nitrate Agar (SCNA) (Küster & Williams, 1964).

Purification of endophytic actinobacteria

Purification of actinobacterial endophytes from mixed populations was carried out to obtain pure strains. The streak plate technique, originally developed by Loeffler and Gaffky in Koch's laboratory, was employed for this purpose (Madigan *et al*., 2018). Actinobacteria were streaked in a quadrant pattern on SCNA plates, and well-isolated single colonies were subsequently selected and re-streaked on fresh SCNA plates to ensure purity.

Maintenance and preservation of endophytic cultures

The purified endophytic actinobacterial isolates were transferred onto SCNA slants. Pure cultures were periodically subcultured on the same medium to maintain viability. All purified actinobacterial isolates were preserved at 4°C until further use.

Characterisation of endophytes

Cultural characterisation

Cultural characteristics were assessed following the methods of the International *Streptomyces* Project (Shirling & Gottlieb, 1966). The colour of aerial and substrate mycelia, along with diffusible pigment production and sporulation—key traits for *Streptomyces* classification—were recorded after cultivating the strains on SCNA at 28°C for 7 days.

Morphological characterisation

Morphological characteristics of the isolates were examined using a compound microscope at 40X and 100X magnifications following the slide culture technique. Thin blocks of SCNA medium were placed on sterile glass slides, transferred into Petri plates lined with moist sterile filter paper, inoculated at the agar edges, and covered with sterile coverslips. The preparations were incubated at 28°C, and after 3-4 days, hyphal arrangement and spore chain morphology were observed. Photomicrographs were obtained using a Cat Cam digital camera (1.3 MP).

Production of endophytic actinobacteria extract

The isolated endophytic cultures were initially cultivated in Starch Casein Nitrate (SCN) broth at 28°C for 7 days. Subsequently, three mycelial discs (6 mm diameter) from actively growing cultures were aseptically transferred to 250 ml Erlenmeyer flasks containing 50 ml of fresh SCN broth. The flasks were incubated at 28°C on a rotary shaker at 180 rpm for 10 days to induce secondary metabolite production. Following incubation, the cultures were centrifuged at 10,000 rpm for 20 min at 4°C to separate the biomass. The resulting cell-free supernatant was collected and used for bioassays to assess antagonistic activity against phytopathogenic fungi.

Determination of antagonistic activity of endophytic actinobacteria extracts

Culture medium

Potato Dextrose Yeast Agar (PDYA) and Potato Dextrose Yeast Broth (PDYB) were used as nutrient-rich media to support optimal growth of fungal phytopathogens in bioassay experiments.

Test microorganisms

The following fungal phytopathogens (Table 1) were isolated in the laboratory and used as test organisms to evaluate the antagonistic activity of endophytic actinomycetes.

Inoculum of pathogenic microbes

The test fungi were grown in potato dextrose broth and incubated at 27°C for 7 days. The resulting spore suspension

Table 1. List of isolated phytopathogens with their respective host and disease

Test microorganisms		
Fungal phytopathogens showed resemblance to the following species	**Host**	**Disease**
Alternaria alternata	*Capsicum annuum*	Fruit rot of chilli
Alternaria brassicicola	*Brassica oleracea var. capitata*	Alternaria leaf spot of cabbage
Alternaria mali	*Malus pumila*	Alternaria blotch of apple
Alternaria tenuissima	*Cajanus cajan*	Alternaria blight of pigeon pea
Colletotrichum capsica	*Capsicum annuum*	Anthracnose of chilli
Colletotrichum gloeosporioides	*Syzygium cumunii* L.	Anthracnose of jamun
Fusarium moniliforme	*Oryza sativa*	Foot rot of rice
Fusarium oxysporum	*Cicer arietinum*	Fusarium wilt of chickpea
Fusarium sacchari	*Saccharum officinarum*	Sugarcane wilt
Ustilaginoidae virens	*Oryza sativa*	False smut of rice
Ustilago tritici	*Triticum aestivum*	Loose smut of wheat

was standardised to approximately 1×10^4 spores/ml for use in the bioassays.

Primary screening of endophytes for anti-phytopathogenic activity

Endophytic filamentous actinobacterial isolates were screened for antifungal activity on Potato Dextrose Yeast Agar (PDYA) medium using a well-diffusion assay. During the primary screening, 200 µl of metabolite extract from each endophytic filamentous actinobacterial isolate was tested against *Colletotrichum capsici*, the causal agent of chilli anthracnose. *C. capsici* was selected as the test pathogen because of its rapid colony development, which facilitated the assessment of antifungal activity within a comparatively shorter incubation period.

Well diffusion assay for determination of antagonistic activity of endophytes (Valgas *et al.*, 2007)

For antifungal screening, 50 ml of molten Potato Dextrose Agar (PDA) was poured into sterile 90 × 15 mm Petri dishes and allowed to solidify. A fungal spore suspension (1×10^4 spores/ml) was evenly spread on the surface and air-dried at room temperature for 30 min. A 6 mm diameter well was aseptically punched into the agar and filled with 200 µl of the endophytic metabolite extract. Plates were incubated at 28°C for 5 days, and antifungal activity was evaluated by measuring the zone of inhibition in four directions from the centre of the well; the mean of these measurements was recorded as the final inhibition zone.

Antagonistic activity of selected endophytic isolates against all test microorganisms

Endophytic isolates demonstrating positive activity in the primary screening were further evaluated for antagonistic effects against all test fungal phytopathogens using the agar well diffusion assay.

RESULTS

Isolation of endophytic actinomycetes

A total of 10 actinobacterial endophytic isolates were isolated from different plant parts (leaves, stem, roots) of various visibly healthy medicinal plants, *viz.*, *Azadirachta indica* and *Datura innoxia*. Results of plant-wise and plant part-wise distribution of endophytic actinobacteria are represented in Table 2 and Figure 1. A maximum of 6 out of 10, *i.e.*, 60% endophytic actinomycetes, were isolated from *D. innoxia,* and the remaining 4, *i.e.*, 40%, were isolated from *A. indica.* The maximum number of isolates were isolated from leaves. Leaves yielded 5 out of 10, *i.e.*,

Table 2. Plant-wise and plant part-wise distribution of endophytic actinomycetes

Plant	Number of endophytic actinobacteria isolated from			Total
	Leaves	Stem	Roots	
Azadirachta indica	2	1	1	4
Datura innoxia	3	2	1	6
Total	5	3	2	10

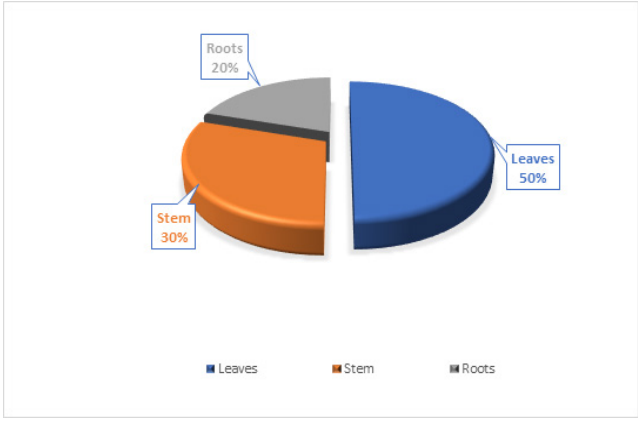


Figure 1. Plant part-wise distribution of endophytic actinobacteria when both plants are taken together.

50% of total isolates, followed by stems, from which 3, *i.e.*, 30% of total isolates. Rest of the 2, *i.e.*, 20% of endophytic actinobacteria, were isolated from roots.

Primary screening of endophytic actinobacterial isolates for antifungal activity

Endophytic actinobacterial isolates were screened for antifungal activity on PDYA medium using a well diffusion assay. In the primary screening, 200µl of metabolite extract of each endophytic filamentous actinobacterial strain was tested for antifungal activity against *C. capsici*, the causal organism of anthracnose of chilli was selected for its characteristic of rapidly growing colonies, which helped to provide results comparatively in a short incubation period. Endophytic isolate DSA-1 showed significant activity, *i.e.*, more than 15 mm, against *C. capsici.* This isolate was selected to determine its antagonist activity against all test organisms.

Morphological characterisation of selected endophytic actinobacterial isolate (DSA-1)

Colonies were regular, raised and hard with pure white aerial mycelium and yellow substrate mycelium. Greenish-

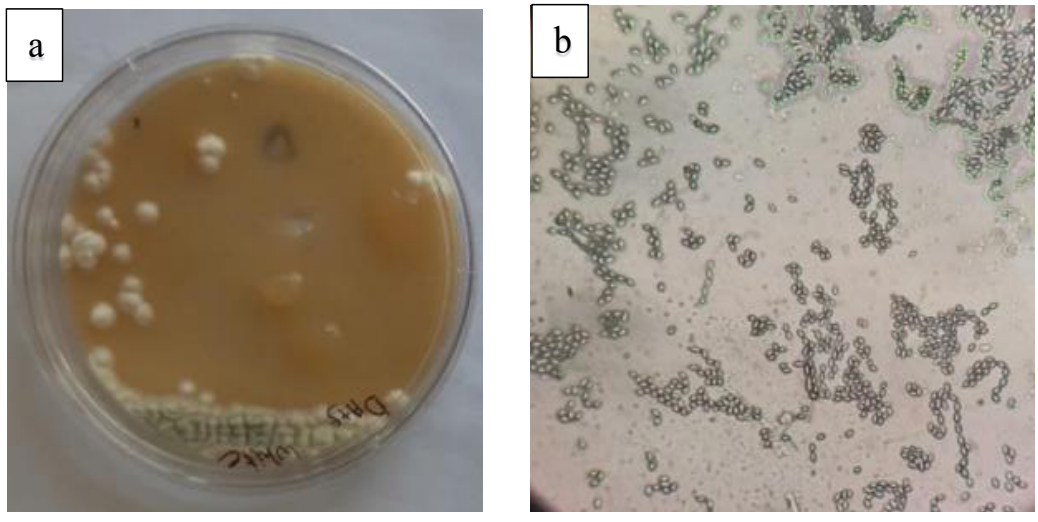


Figure 2. Selected actinobacterial isolate DSA-1. **(a).** Colony morphology and **(b).** microscopic observation.

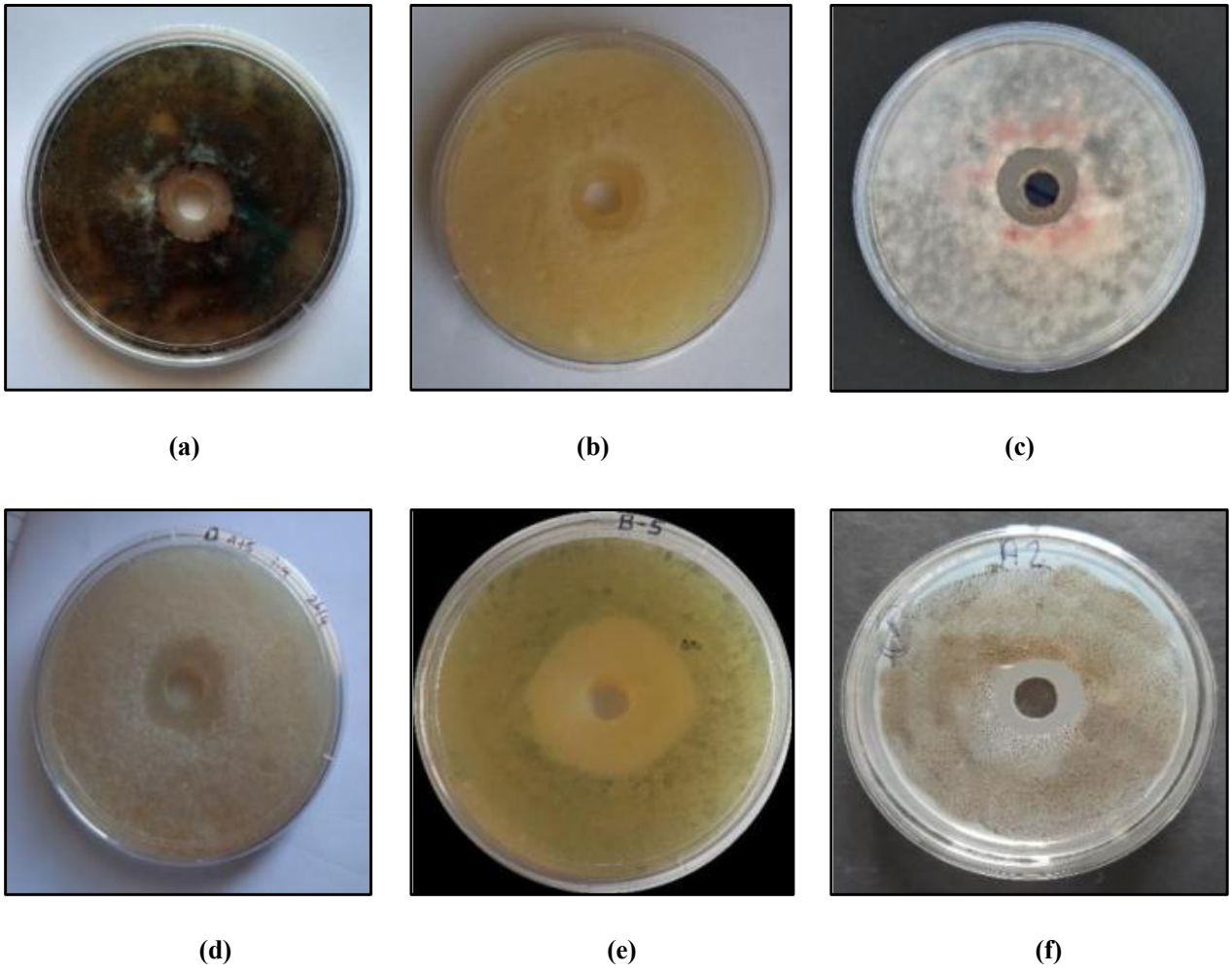


Figure 3. Antagonistic activity (Zone of inhibition) of DSA-1 (*Streptomyces* sp.) against various fungal phytopathogens: **(a).** *Alternaria alternaria*, **(b).** *Colletotrichum capsici*, **(c).** *Fusarium moniliforme*, **(d).** *Fusarium sacchari*, **(e).***Ustilaginoidae virens* and **(f).** *Ustilago tritici*.

blue sporulation was observed on aerial mycelium. A simple slide was prepared from a well-isolated single colony and was observed under a microscope. Scattered round spores and chains of spores were observed, as shown in Figure 2. The isolate shows resemblance to one of the *Streptomyces* sp. Zhao *et al.* (2019) reported the same morphology of one of the *Streptomyces* sp**.**

Determination of antagonistic activity of selected endophytic actinomycetes isolates against various phytopathogens

Selected isolate DSA-1 (*Streptomyces* sp.) was evaluated for its antifungal activity against 11 agriculturally important fungal phytopathogens using a well diffusion assay. The amount of metabolite extract of the selected isolate was kept at 200 µl for all test organisms.

Results of the antagonistic activity of the selected filamentous actinobacterial isolate were represented in Table 3. It can be inferred from the table that Endophytic isolate DSA-1, *i.e. Streptomyces* sp**.**, showed significant activity against 6 out of 11 test organisms used in the study. Maximum activity of DSA-1 was observed against *Ustilaginoidae virens*, the causal organism of false smut of rice (22.8 mm), followed by *Fusarium sacchari*, the causal organism of sugarcane wilt (12.4 mm), *Ustilago tritici,* the causal organism of Loose smut of wheat (12.3 mm) and *Alternaria alternata,* the causal organism of Fruit rot of

Table 3. Antagonistic activity of selected isolate DSA-1 (*Streptomyces sp.*) against fungal phytopathogens

S. No.	Test microorganisms (Fungal phytopathogens)	Zone of inhibition exhibited by selected endophytic isolates (mm)
1	*Alternaria alternata*	12.2
2	*Alternaria brassicicola*	-
3	*Alternaria mali*	-
4	*Alternaria tenuissima*	-
5	*Colletotrichum capsica*	12
6	*Colletotrichum gloeosporioides*	-
7	*Fusarium moniliforme*	12
8	*Fusarium oxysporum*	-
9	*Fusarium sacchari*	12.4
10	*Ustilaginoidae virens*	22.8
11	*Ustilago tritici*	12.3

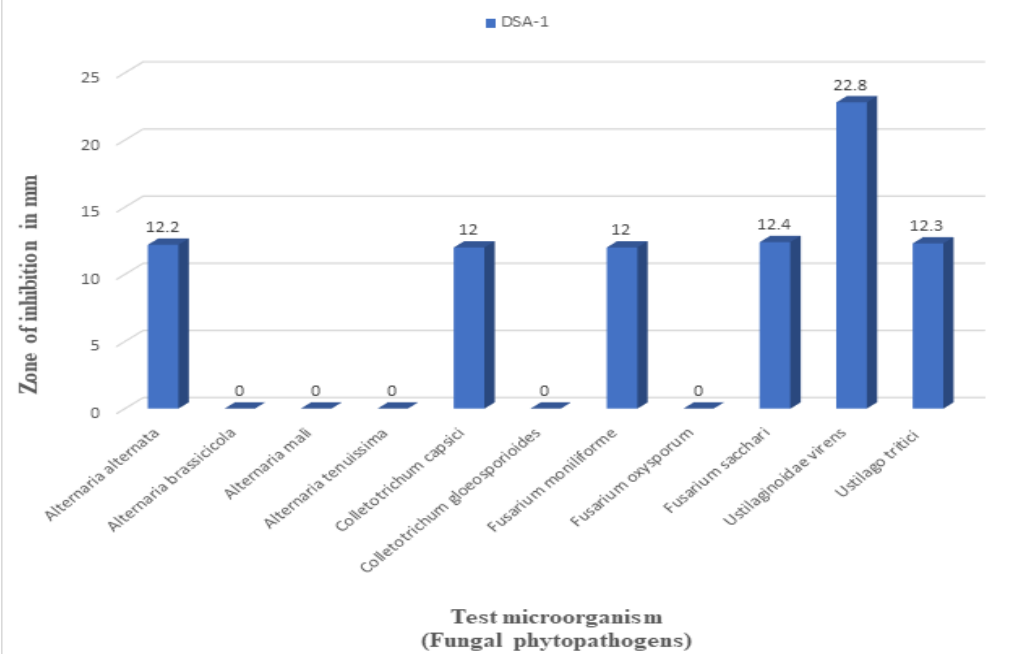


Figure 4. Bar graph representation of the antagonistic activity of selected isolate DSA-1 (*Streptomyces sp.*) against fungal phytopathogens.

chilli (12.2 mm). Endophytic isolate DSA-1 did not show activity against *Alternaria brassicicola*, *Alternaria mali*, and *Alternaria tenuissima*. *Colletotrichum gloeosporioides* and *Fusarium oxysporum*.

DISCUSSION

Plant-associated endophytic actinobacteria, particularly those isolated from medicinal flora, represent an invaluable reservoir of novel bioactive compounds

with potential applications in sustainable agriculture and biological disease control (Zou *et al*., 2023; Mohamad *et al*., 2024). In the present study, ten endophytic actinomycetes were successfully isolated from the medicinal plants *Azadirachta indica* and *Datura innoxia*. A tissue-specific distribution of the isolates was observed, with leaf tissues yielding the highest proportion of isolates (50%, n = 5/10). This pronounced preference for foliar tissues suggests that the leaf microenvironment of *A. indica* and *D. innoxia*, characterised by the availability of diverse primary and secondary metabolites, may provide a favourable ecological niche for the establishment and persistence of endophytic actinobacteria. These findings are consistent with Wang *et al.* (2023), who isolated *Streptomyces* sp. NEAU-ZSY13 from the leaf tissue of *Perilla frutescens*, with foliar tissues similarly accounting for a substantial proportion of the total isolates (24 of 76 isolates). Likewise, Zou *et al.* (2023) demonstrated that medicinal plants such as *Aconitum carmichaelii* harbour rich reservoirs of culturable endophytic actinobacteria. However, the predominance of leaf-derived isolates observed in the present study contrasts with reports in which root tissues exhibited higher isolation frequencies than leaves (Wang *et al*., 2023). Such differences in tissue colonisation patterns among studies may be attributed to variations in host physiology, tissue architecture, plant-associated chemical profiles, environmental exposure, and the specific isolation media employed (Zou *et al*., 2023; Wang *et al*., 2023). The relatively higher recovery of actinomycetes from leaves in the present study therefore highlights foliar tissues as a promising niche for the targeted isolation and bioprospecting of endophytic actinobacteria from medicinal plants.

In the antagonism assay, isolate DSA-1 exhibited a broad antifungal spectrum, inhibiting 6 of the 11 phytopathogenic fungi tested. The inhibited pathogens included *Alternaria alternata*, *Colletotrichum capsici*, *Fusarium moniliforme*, *F. sacchari*, *Ustilaginoidea virens*, and *Ustilago tritici*, whereas no visible inhibition was observed against *A. brassicicola*, *A. mali*, *A. tenuissima*, *C. gloeosporioides*, and *F. oxysporum*. The ability of endophytic actinomycetes, particularly *Streptomyces*, to suppress phytopathogenic fungi has been reported previously, supporting the antagonistic potential observed for DSA-1. Liu *et al.* (2019), for example, reported that the endophytic *Streptomyces* sp. NEAU-S7GS2 strongly inhibited the mycelial growth of *Sclerotinia sclerotiorum*, *Exserohilum turcicum*, *Rhizoctonia solani*, and *Corynespora cassiicola*, with reductions in mycelial growth ranging from 57.9 to 99.1%. Importantly, their study further demonstrated that the cell-free fermentation supernatant and methanol extract of the mycelia retained antifungal activity, suggesting that diffusible antifungal substances contributed to the antagonistic effect of the strain. Although the present study did not characterise the metabolites responsible for DSA-1 activity, the broad inhibition observed against six fungal pathogens similarly suggests that DSA-1 may produce diffusible or biologically active compounds capable of interfering with fungal growth.

The broad-spectrum activity observed for DSA-1 is also comparable to the biocontrol potential reported for other endophytic *Streptomyces*. Goudjal *et al.* (2016) evaluated sixteen endophytic actinobacteria and identified *Streptomyces* sp. SNL2 as a promising antagonist against *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The isolate exhibited several traits associated with biological control, including production of siderophores, hydrogen cyanide, chitinase and β-1,3-glucanase, and subsequently provided substantial protection of tomato seedlings against *Fusarium* root rot. These findings indicate that the antagonistic potential of endophytic actinobacteria may involve multiple complementary mechanisms rather than a single antifungal factor. Similarly, El-Akshar *et al.* (2025) reported strong antifungal activity in endophytic *Streptomyces enissocaesilis* and *Streptomyces rochei* against *F. solani* and *R. solani*, with marked reduction of fungal mycelial growth. Their microscopic observations further showed degradation of fungal mycelia, while greenhouse experiments demonstrated suppression of disease and improved plant performance. In comparison, DSA-1 inhibited *F. moniliforme* and *F. sacchari* but did not inhibit *F. oxysporum*. Such differences in pathogen susceptibility are not unexpected, as antifungal activity can vary according to the fungal species and the antimicrobial compounds produced by individual actinomycete isolates.

The selective inhibition pattern observed in the present study is therefore an important characteristic of DSA-1 rather than evidence of a lack of antagonistic potential. DSA-1 inhibited fungi belonging to several taxonomically distinct genera, including *Alternaria*, *Colletotrichum*, *Fusarium*, *Ustilaginoidea*, and *Ustilago*, indicating activity

across a relatively diverse group of phytopathogens. At the same time, the absence of inhibition against five tested pathogens demonstrates that its activity was not uniformly effective against all fungi. Differences in fungal susceptibility may be related to variation in fungal cell-wall composition, membrane properties, detoxification capacity, or the ability of individual pathogens to tolerate or neutralise antimicrobial metabolites. However, these mechanisms were not directly investigated in the present study and therefore require experimental confirmation. Taken together, the inhibition of six fungal phytopathogens by DSA-1, together with the broad antifungal and biocontrol activities previously reported for endophytic *Streptomyces* (Liu *et al*., 2019; Goudjal *et al*., 2016; El-Akshar *et al*., 2025), indicates that DSA-1 represents a promising candidate for further investigation. Future studies involving cell-free culture filtrates, solvent extracts, metabolite profiling, and molecular characterisation would help determine whether its observed antagonism is associated with diffusible secondary metabolites, hydrolytic enzymes, or other antifungal mechanisms.

Among the endophytic actinomycetes isolated in the present study, isolate DSA-1 exhibited morphological characteristics that resembled those commonly associated with the genus *Streptomyces*. Based on microscopic observation of its mycelial growth and spore characteristics, DSA-1 was therefore considered *Streptomyces*-like in the present study. However, this observation represents a preliminary morphological characterisation, and molecular identification would be required to confirm its taxonomic position. The occurrence of a *Streptomyces*-like isolate among the endophytes recovered from *Azadirachta indica* and *Datura innoxia* is consistent with previous reports of endophytic *Streptomyces* associated with medicinal plants. For example, Wang *et al.* (2023) reported an endophytic *Streptomyces* sp. NEAU-ZSY13 isolated from the leaf tissue of the medicinal plant *Perilla frutescens*. Similarly, Zou *et al.* (2023) investigated culturable endophytic bacteria associated with the medicinal plant *Aconitum carmichaelii*, demonstrating the presence of diverse endophytic bacteria with antimicrobial and plant-growth-promoting potential. Mohamad *et al.* (2024) further reported an endophytic *Streptomyces albidoflavus* associated with the medicinal plant *Thymus roseus* and demonstrated its capacity to produce bioactive metabolites with antifungal and plant-growth-promoting effects. Thus, the morphological resemblance of DSA-1 to *Streptomyces*, together with the occurrence of endophytic *Streptomyces* reported from other medicinal plants, supports its consideration as a potentially important actinomycete isolate.

CONCLUSION

Overall, the present findings demonstrate that endophytic actinomycetes associated with the medicinal plants *Azadirachta indica* and *Datura innoxia* represent a potentially useful source of fungal antagonists. The higher recovery of isolates from leaf tissues suggests that foliar tissues may provide a favourable niche for endophytic actinomycetes and may therefore be considered in future bioprospecting efforts. Among the isolates obtained, DSA-1 showed the most notable antagonistic potential, inhibiting six of the eleven phytopathogenic fungi tested and exhibiting activity against pathogens belonging to five different fungal genera.

The morphological characteristics of DSA-1 were consistent with those commonly observed in *Streptomyces*-like actinomycetes; however, this observation alone is insufficient for definitive taxonomic identification. Therefore, DSA-1 should be regarded as a *Streptomyces*-like isolate until its identity is confirmed through molecular characterisation. Its antifungal activity makes it a promising candidate for further investigation, particularly through studies of cell-free culture filtrates, solvent extracts, secondary metabolite profiles, and antifungal mechanisms. Molecular characterisation and subsequent greenhouse and field evaluation would also be necessary to determine its taxonomic identity, biological efficacy, consistency of antagonistic activity, and practical potential as a biological control agent. Thus, the present study provides a useful preliminary basis for further exploration of medicinal-plant-associated endophytic actinomycetes as potential sources of environmentally compatible alternatives for the management of fungal phytopathogens.

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AUTHOR CONTRIBUTIONS

Jatinder Singh: Conceptualisation, methodology, investigation, data collection, data analysis, and writing original draft preparation. Bahaderjeet Singh: Supervision, guidance, validation, and review of the manuscript. Gurjant Singh: Assistance in manuscript editing. Dr. Pankaj: Data analysis, revision of the draft and final preparation of the manuscript. All authors read and approved the final manuscript.