



Biocontrol activity of *Streptomyces* isolate SDSRO-13 against *Colletotrichum* spp. causing anthracnose disease of chilli (*Capsicum annuum* L.)

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Abstract

A total of 18 *Streptomyces* species were isolated from the rhizospheric soil of maize. Among 18 isolates, SDSRO-13 showed strong inhibition against *Colletotrichum* spp. of chilli. SDSRO-13 was identified as *Streptomyces* sp. based on morphological, biochemical and 16S rRNA partial gene sequence analysis. In this study, two strains of *Colletotrichum* sp. were used for pathogenicity test. For *in vivo* antifungal activity, spore suspension of the test pathogens were used on the fresh chilli fruit by making a small puncture on the fruit, in completely sterile conditions and incubated for up to 21 days. The chilli fruits treated with SDSRO-13 and test pathogens showed no disease symptoms. The infected fruits were subjected for Koch's postulate test and they showed the similar morphological features compared to the pure cultures of the pathogens. We conclude that actinomycetes, especially members of the genus *Streptomyces* are potential biocontrol agents which offer safer alternative management strategy to control the chilli fungal pathogens.

Keywords: Actinomycetes, 16S rRNA, *Colletotrichum* sp., chilli, anthracnose disease, biocontrol activity

Introduction

There are many difficulties in modern agriculture, particularly in developing nations where rising population increase the demand for food grains and the necessity of trade and economic growth increases the demand for diverse cash crop goods. Chilli (*Capsicum annuum* L.) is regarded as the most significant vegetable and spice crop in the entire globe. It is a member of the *Solanaceae* family and belongs to the genus *Capsicum*. India is the largest producer, consumer and exporter of chillies worldwide. Chillies also have significant health benefits due to their antioxidant and anti-inflammatory qualities (Zhuang *et al.*, 2020). The rise of several phytopathogens, which pose a severe danger to productivity and the quality of the goods produced, is another difficulty modern agriculture is facing. The use of fertilizers and chemical agents like fungicides, insecticides, and other pesticides significantly reduces these issues. Excessive application of chemicals in agricultural fields has negative impacts on crops and environment. Therefore, it is vital to adopt eco-friendly techniques to reduce dumping of these toxic compounds to the soil. The beneficial bacteria in the soil must be reintroduced in order to restore the soil's natural fertility (Jisha *et al.*, 2018).

More specifically in the tropical and subtropical regions, phytopathogenic fungi pose significant challenges to the cultivation of economically valuable plants. Many vegetable and fruit products suffer from infections caused by phytopathogenic fungi that emerge during crop development or after harvest. Additionally, the

phytopathogenic fungus creates toxic compounds that pose a threat to public health since they have the potential to cause cancer. On the other hand, the growth of some fungi causes nutritional and chemical changes, undesirable appearance, and the creation of food flavour (Vashistha and Chaudhary, 2019). The four major plant pathogens in the world—*Fusarium*, *Botrytis cinerea*, *Alternaria alternata*, and *Colletotrichum* spp.—cause the economically significant disease anthracnose in a variety of hosts, including grains, legumes, vegetables, and tree crops. Additionally, they are most prevalent in agriculture and have the most economic impact. An important crop with a global economic impact, chilli (*Capsicum* spp.) is one of these hosts and is badly affected by the anthracnose disease, which can result in yield losses of up to 50% (Pakdeevaporn *et al.*, 2005). Anthracnose of chilli (*Capsicum annuum* L.) produces pink to orange conidial (spores) masses that appear as dark brown to black sunken patches, round or angular shapes, and concentric rings of acervuli that are frequently damp. When chilli fruits are mature, this disease is really bad. The pathogen spreads by seeds, soil, and air (Veerendra *et al.*, 2017). To combat microbial diseases, chemical insecticides and antibiotics have been employed. However, the usage of these compounds has resulted in a variety of issues, including soil salinization, environmental contamination, and disease resistance to treatment (Petriccione *et al.*, 2017; Rodriguez *et al.*, 2020).

In India and many other nations in Southeast Asia and Oceania, the

Colletotrichum species *C. capsici*, *C. gloeosporioides*, and *C. acutatum* are known to cause the anthracnose disease of the chilli plant. In Southern India, *Colletotrichum capsici* and *C. gloeosporioides* were the two most common species. In accordance with Reddy *et al.* (2014), *C. capsici* was widespread in Karnataka, Tamil Nadu, and Maharashtra.

The genus *Streptomyces* was initially described by Waksman and Henrici in 1943 (Waksman and Henrici. 1943). The structure and chemical nature of cell wall are employed to place it in the Streptomycetaceae family. According to an earlier research, the *Streptomyces* sp. is responsible for the production of more than 74% of the antibiotics used today. Gram-positive filamentous soil bacteria *Streptomyces* sp. produces a wide range of secondary metabolites, including various useful chemicals including enzymes, antibiotics, and volatile organic molecules. Moreover, it increases the disease resistance of plants (Sandra Pacio *et al.*, 2021). *Streptomyces* species have garnered a lot of attention in the field of biological control of soil diseases due to their substantial synthesis of secondary metabolites. The production and release of secondary metabolites depend on several factors, such as nutritional, biological, and environmental conditions. Furthermore, they can be induced or activated by other organisms when they invade the soil space, competing for available nutrients (Sun *et al.*, 2017).

Rhizobacteria that promote plant growth can be used as both biofertilizers and biocontrol agents, which can be very

beneficial. Because they can produce a variety of bioactive substances that are poisonous to phytopathogens but not to humans or the environment. Actinomycetes have a unique ability to prevent the growth of a wide range of bacterial and fungal phytopathogens. Because of these traits, actinomycetes are a preferred substitute for chemical applications (Sabaratnam and Traquair, 2015).

Materials and methods

Collection of soil sample

The rhizosphere soil sample was collected from maize field at Harapanahalli taluk (14.7734076 N, 75.985134 E), Vijayanagar (district), Karnataka (state), India. The soil was collected in a sterile polythene ziplock bag from a depth of about 15-20 cm and brought to the laboratory, dried and treated with 1% CaCO₃ for further study.

Isolation of actinomycetes

The soil sample was subjected to serial dilution from 10⁻¹ to 10⁻⁶ and plated on Starch Casein Nitrate Agar (SCNA), Oatmeal agar, Kenknight agar and Actinomycetes Isolation Agar media amended with fluconazole [antibiotic to control fungal growth]. The plates were incubated at 30° C for 10-15 days. Streak plate method was used to purify the actinomycetes colonies. A total of 18 actinomycetes isolates were obtained. All the 18 isolated colonies were subcultured on SCNA slants and preserved at 4°C for further analysis. Various biochemical tests were performed as stated by Mesta and Onkarappa, (2017).

Primary screening of antifungal activity of *Streptomyces* spp

The primary screening of antifungal activity of all the 18 *Streptomyces* isolates were carried out by cross streak method on SCN media. The media was amended with peptone and dextrose to facilitate fungal growth. The plates were prepared and the *Streptomyces* isolates were inoculated by a single line streak in the centre of the petriplate and were incubated for four days at 30±2°C. After 4 days the plates were inoculated with the test organisms perpendicular to the growth of actinomycetes isolates and incubated for 72 hours for fungal growth. The absence of growth or a less dense growth of test organism near the actinomycetes isolate was considered positive for production of antifungal metabolite by the isolates (Sahin and Ugur. 2003; Haritha *et al.*, 2010).

Secondary screening of *Streptomyces* sp. by dual culture assay

Antifungal activity of *Streptomyces* sp. SDSRO-13 against chilli pathogen was evaluated by using dual culture assay. Actively growing fungal mycelia of *Colletotrichum* strains were inoculated on the modified SCN media at a distance of 1.5 cm. The *Streptomyces* isolate SDSRO-13 was inoculated 4 days prior to the fungal inoculation. All the plates were incubated at 25±2°C for 7-14 days (Tatsuya *et al.*, 2018). After incubation, the zone of inhibition was measured and colony growth inhibition (%) was calculated by using the formula: $PI = \frac{C-T}{C} \times 100$, where PI is the percent inhibition, C is the colony growth of the pathogen in control, and T is the colony growth of

pathogen in dual culture (Shrivastava *et al.*, 2017).

Morphological and staining characteristics of actinomycetes-SDSRO-13

The potent isolate SDSRO-13 was identified and confirmed as *Streptomyces* sp. as per the identification criteria in Bergey's Manual of Systematic Bacteriology, Systematic bacteriology and the International Streptomyces Project guidelines. The isolate was found to be Gram positive and non acid fast. Various biochemical test results were reported (Mesta and Onkarappa. 2017).

Molecular characterization of SDSRO-13

The isolate SDSRO-13 was subjected for 16S rRNA sequencing studies and phylogenetic analysis to identify the potent isolate to its nearer species. The molecular characterization studies were carried out at NCIM (National Centre for Industrial Microorganisms), Pune, Maharashtra, India.

DNA isolation: The isolation of DNA from the culture was carried out using Spin column kit from CSIR- National Chemical Laboratory.

Primer used was **16S rRNA 704F (757bp)**

Forward primer: 5' CGAAAGTCGGTAACA CCCGA 3'

Reverse primer: 3' CTCCGGTACGGCTAC CTTG 5'

For PCR amplification of 16S rRNA gene was amplified using polymerase chain reaction in a thermal cycler and were purified using Exonuclease I-Shrimp Alkaline Phosphatase (Exo-SAP) (Darby *et*

al., 2005). Purified amplicons were sequenced by Sanger method in ABI 3500xL genetic analyzer. Sequencing files edited using CHROMOSLITE (version 1.5) and further analyzed by Basic Local Alignment Search Tool (BLAST) with closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI) database that finds regions of local similarity between sequences (Altschul *et al.*, 1990).

Isolation of *Colletotrichum* sp. from anthracnose infected chilli fruit

Colletotrichum infected chilli fruits were brought to the laboratory in a sterile polythene bag. The sample was surface sterilized with 0.2% sodium hypochlorite solution followed by distilled water 4-5 times to complete removal of sodium hypochlorite solution. With the use of a sterilized blade, small sections of contaminated tissue (2–3 mm in length) were cut at the intersection of the sick and healthy portions. In aseptic conditions with laminar air flow, these infected parts were surface sterilized with 0.2% sodium hypochlorite solution for 30 seconds, followed by 3–4 times washing with sterilized distilled water. These pieces were placed on sterile blotting paper for drying. Later, five bits were placed aseptically on PDA plates, incubated at 25±2°C for 5-7 days, and checked often to monitor the fungus' growth as it emerged from various sections. The fungal isolates underwent additional purification by sub culturing, and they were then kept on PDA slants at 4° C for further examination (Pavithra *et al.*, 2019).

Molecular characterization of the isolated *Colletotrichum* pathogen

After isolation and purification the pathogen was identified based on morphological, cultural and molecular characters. Chromosomal DNA was extracted by using Spin column kit. The ITS region was amplified using universal primers and Taq DNA polymerase. The PCR amplicon obtained was subjected to ExoSAP purification, sequenced and subjected to BLAST analysis. Each isolate was reported with the first five-ten hits observed in the database. Further multiple sequence alignment and phylogenetic analysis were carried out for accurate species prediction and evolutionary relationship (Karlin *et al.*, 1990; Myers *et al.*, 1988).

Primer sequence:

ITS 1	TCC GTA GGT GAA CCT GCG G
ITS 4	TCC TCC GCT TAT TGA TAT GC

Pathogenicity test

Healthy chilli fruits were brought to the laboratory from nearby market and were surface sterilized with 0.2% sodium hypochlorite for 30 seconds, washed with sterilized distilled water for about 4-5 times for the complete removal of hydrogen peroxide. Sterilized chilli fruits were air dried in laminar air flow by placing on sterilized blotting paper and a small puncture was made on the fruit with sterilized needle and was inoculated with spore suspension of purified pathogens of *Colletotrichum capsicum* and *Colletotrichum* sp. The inoculated fruits with punctured, without punctured and treated with

sterilized distilled water were placed on moist chamber with double layered sterilized blotting papers and kept for incubation at $25\pm 2^\circ\text{C}$ (Zee & Vu. 2018). After 5 days, the chilli fruits were evaluated for any infection by the pathogens. Tests were conducted in triplicates (Jeffrey *et al.*, 2015).

Efficacy of SDSRO-13 against anthracnose of chilli

The pathogens were inoculated 3 days after the actinomycetes growth to allow the production of bio-active compounds (Kunova *et al.*, 2016). After 4 days, about 10 mm disc of actively growing pathogen culture was placed on modified SCNA media, 1 cm away from the edge of Petri dish, opposite to the previously inoculated Actinomycetes isolate in a sterile conditions. Modified SCNA media inoculated with the pathogens alone served as control. The plates were incubated at $28\pm 2^\circ\text{C}$. When the control plates showed full growth of the pathogen the radial growth of the mycelium was measured. The results were expressed as per cent inhibition of growth over control (Saputri *et al.*, 2021). The percent inhibition growth was calculated by, $I = C - T / C \times 100$. Where I = Inhibition percentage; C = Colony diameter in control (mm); T = Colony diameter in treatment.

Thin layer chromatography

Solvent extraction of metabolite

SDSRO-13 isolate was bulk-cultivated for 10 days using SCN broth, after which the broth was filtered through Whatman Grade-01 filter paper. The metabolite was extracted by mixing ethyl acetate and culture filtrate in

1:1 proportion, the mixture was vigorously agitated in a separating funnel and the funnel was allowed to stand undisturbed for 30-45 min. Later, broth layer was separated carefully avoiding mixing of separated fractions and the solvent layer was evaporated at 40°C . The pale pink resinous mixture obtained was tested for TLC (Augustine *et al.*, 2005a).

The crude extract was resolved for its components by TLC with methanol: water (9:1) solvent system. The chromatograms were allowed to air dry, and were exposed to iodine vapours for the detection of compounds. The slides were observed for separation and appearance of metabolite components (Augustine *et al.*, 2005a; Rahman and Humainy, 2011).

Preparation of fungal inoculum

Potato Dextrose Broth was prepared and sterilized for the cultivation of fungal cultures. Loopful of fungal spores were inoculated to the media and were incubated at room temperature for 48-72 hours for luxuriant growth.

Well in agar method

The secondary screening was carried out by well in agar method. The 24-72 hours old *Colletotrichum* fungal cultures were swab inoculated onto the PDA plates. 8 mm wells were made using sterile cork borer; 300 μl of culture filtrate, solvent extract and solvent (control) were added to the wells. The plates were incubated in upright position at room temperature for 48-72 hours and the plates were observed for antifungal activity of the metabolite measured as zone of inhibition

(mm) after incubation using antibiotic zone measurement scale (HiMedia). The experiment was carried out in triplicates.

Results and discussion

Isolation of actinomycetes

Total of 18 actinomycetes species were isolated from the rhizosphere soil of maize. For the isolation of *Streptomyces* sp. SCNA, Oatmeal agar, Kenknight agar and Actinomycetes Isolation Agar media were used. Among the 4 media, SCNA was the best medium as it yielded the highest number of actinomycetes.

Sharma and Manhas (2020) reported the *Streptomyces* isolate M4 showed good

growth on different media. Microscopic studies revealed the formation of branched substrate mycelium and aerial hyphae having retinaculum coiled spore chains bearing smooth surface spores. The isolate hydrolyzed starch, cellulose, esculin, urea, lipid and gelatin but did not show casein hydrolysis.

Primary screening for antifungal activity of *Streptomyces* spp.

The primary screening of the *Streptomyces* isolates revealed antifungal activity against the test fungi to a varied extent. The isolate SDSRO- 13 showed broad spectrum antifungal activity inhibiting all the six fungal pathogens of chilli (Fig.1).

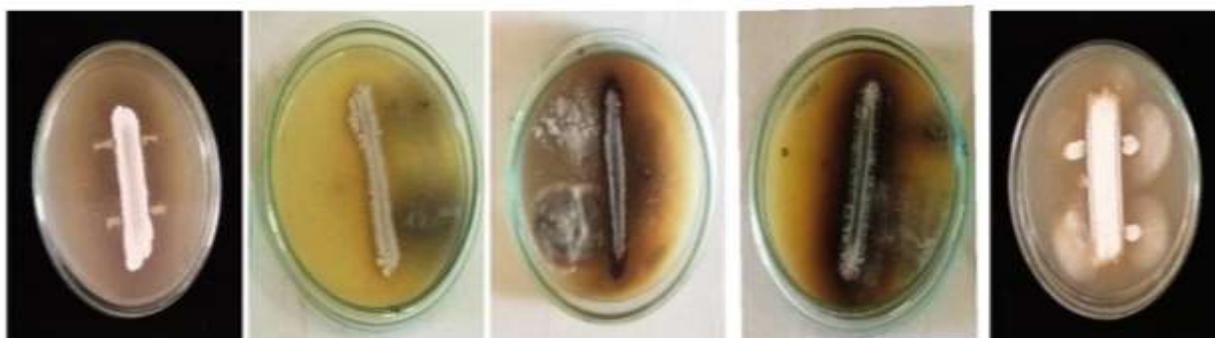


Fig. 1. Primary screening of antifungal activity of *Streptomyces* spp. against fungal pathogens of chilli

Secondary screening of *Streptomyces* sp. by dual culture assay

Secondary screening of antifungal activity by dual culture method was performed only

with the potent *Streptomyces* isolate SDSRO-13 against the *Colletotrichum* spp. The isolate SDSRO-13 showed maximum inhibition of *Colletotrichum* mycelial growth which was tested after 7 days of incubation (Fig. 2).



Fig. 2. *In vitro* & *in vivo* biocontrol assay of SDSRO-13 against *Colletotrichum* pathogens of chilli

Morphological and staining characteristics of SDSRO-13

Morphological and staining characteristics of SDSRO-13 is given in table 1. Similar

findings were obtained by Mesta and Onkarappa (2017).

Table 1. Staining, biochemical characters and spore arrangement

Tests	Characteristics
Gram's staining	+
Acid fast staining	-
Starch hydrolysis	+
Gelatin hydrolysis	+
Casein hydrolysis	+
Catalase test	+
H ₂ S production	-
Spore arrangement	Rectus

Molecular characterization of SDSRO-13

Streptomyces sp. SDSRO-13 was identified to be *S. rochei* according to its 16S rRNA gene sequence. The partial 16S rRNA sequences (757bp) for the strain SDSRO-13 was

obtained. Phylogenetic analysis based on the NCBI gene sequences indicated that the organism is a *Streptomyces* sp. The isolate showed close homology to *Streptomyces rochei* (Fig.3).

Molecular characterization of SDSRO-13

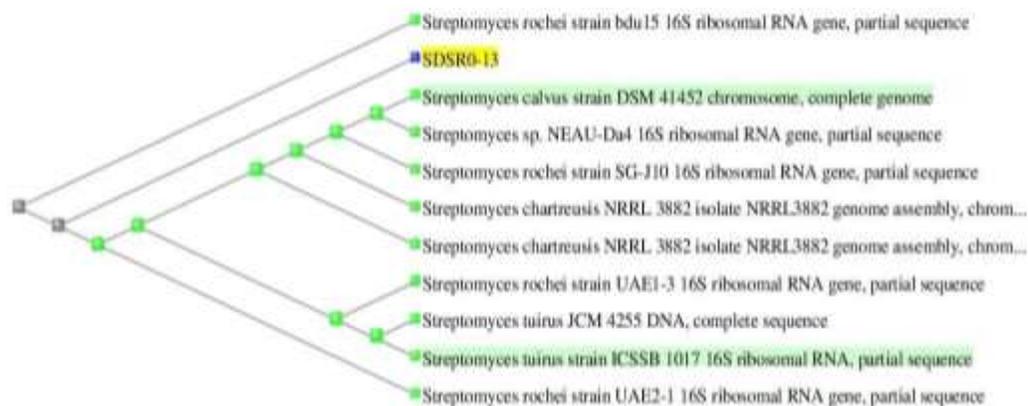


Fig. 3. Phylogenetic tree showing the position of *Streptomyces* sp. SDSRO-13 on the basis of 16S gene sequence analysis.

According to Mohammadipناه and Wink (2016), a variety of mechanisms, including competition for iron via the synthesis of siderophores, the production of antibiotics, and the secretion of lytic enzymes like chitinases, β -1,3-glucanases, lipases, cellulases, and exoproteases, mediate the inhibition of mycelia growth. The culture filtrate of the isolate significantly reduced the growth of *C. capsici* and the onset of anthracnose disease on the artificially inoculated chilli fruits. Earlier investigations by Veerendra *et al.* (2017) isolate SDSRO-13 produced compounds that have an antifungal impact.

Isolation of *Colletotrichum* sp. from anthracnose infected chilli fruits

Circular or angular deep lesions with concentric rings of acervuli that usually develop pink to orange conidial masses and have moist traits are typical fruit symptoms. *Colletotrichum* species produced acervuli that were sickle-shaped, waxy, sub epidermal, and often covered in dark needle-like setae (Veerendra *et al.*, 2017). The

findings showed that *Colletotrichum* sp. was recognized following purification based on colony colour, growth pattern, and pattern of acervuli development on PDA (Fig. 4). Zee and Vu. (2018) have reported similar findings (2018).



Fig. 4. Pure cultures of *Colletotrichum* strains.

Under *in vitro* conditions, the pathogenicity of both *Colletotrichum* strains was demonstrated by Koch's postulates. Under *in vivo* condition healthy fruits inoculated with the phytopathogen showed mycelial growth and symptoms of anthracnose. The phytopathogen was re isolated from the infected fruits and compared with the original culture and found to be the same.

Fruits inoculated with sterile water which served as control did not show the symptoms of disease (Linu *et al.*, 2017).

Morphological characterization of the *Colletotrichum* sp.

Following isolation and purification, *Colletotrichum* species were recognized based on colony colour and growth pattern on PDA media. When viewed under a microscope, the separated *Colletotrichum* sp.

produced round, fluffy mycelium that was predominantly white to light mouse grey in colour and had older, black-colored acervuli that spread over the culture. The spores of both strains were sickle shaped with tapering ends and larger in case of isolated *Colletotrichum* sp. compared to *C. capsici* and the spores were aseptate in nature. The hyphae of both the strains were coiled and septate (Table 2) (Fig. 5).

Table 2. Colony morphology of the *Colletotrichum* spp.

Name of the pathogen	Color of the colony on PDA	Conidial shape	Size of the Conidia	Nature of hyphae
<i>Colletotrichum</i> sp.	Mouse gray (light orange- back view)	Sickle -aseptate	Large	Septate
<i>C. capsici</i>	White (black blue-back view)	Sickle- aseptate	Medium	Septate



Fig. 5. Microscopic view of *Colletotrichum* spores

Molecular characterization of the *Colletotrichum* sp.

The isolate's nucleotide homology and phylogenetic analysis revealed an undeniable similarity to *C. truncatum* (Fig.

6). For the first time, Mills *et al.* (1992) employed DNA sequence data to discriminate among various *Colletotrichum* species, and they discovered variation in the ITS1 region of nrDNA (nuclear ribosomal DNA).

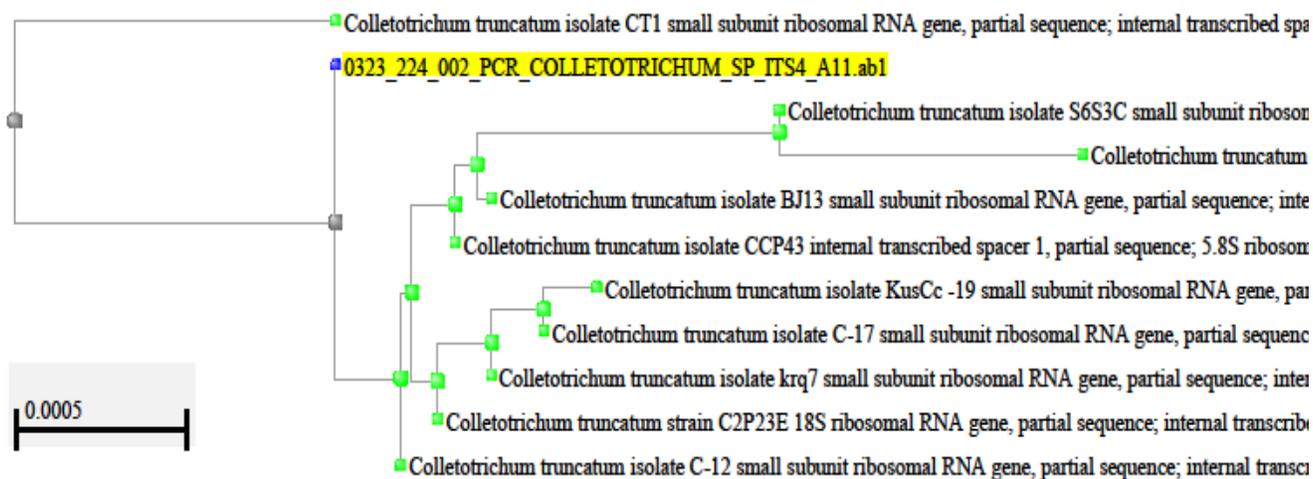


Fig. 6. Molecular characterization of *Colletotrichum* sp.

Similar work was carried out by Hassan *et al.* (2018), the isolates were identified based on the obtained ITS, ACT and GAPDH gene sequences. A BLAST search resulted in sequences from two *Colletotrichum* spp. of >99% sequence similarity: *C. siamense* and *C. horii*. The isolates ICK-3, ICK-22, ICK-23, and ICK-47 were identified as *C. siamense* and ICK-84, ICK-91, ICK-103, and ICK-111 as *C. horii*. Although the ITS sequences of ICK-3 and ICK-22 matched with undefined *Colletotrichum* spp. and ICK-47 matched with *C. gloeosporioides* their (ICK-3, ICK-22, ICK-23, and ICK-47) ACT and GAPDH gene sequences were most similar to those of *C. siamense* isolates (BMLI15, LQ22, Xt-18-1, and LC3049).

Percentage inhibition of SDSRO-13 against *Colletotrichum* spp. of chilli

The isolate SDSRO-13 suppressed the growth of both the strains of the *Colletotrichum* sp. Up to 90% inhibition was observed against isolated *Colletotrichum* sp. and 71% in case of *C. capsici* (Fig.7). Similar

findings were also reported by Hyi *et al.* (2014). The crude cell free culture suspension of 12 days old broth of the isolate SDSRO-13 suppressed the growth of both the *Colletotrichum* strains. The crude sample had antifungal activity (Jeffrey *et al.*, 2015).

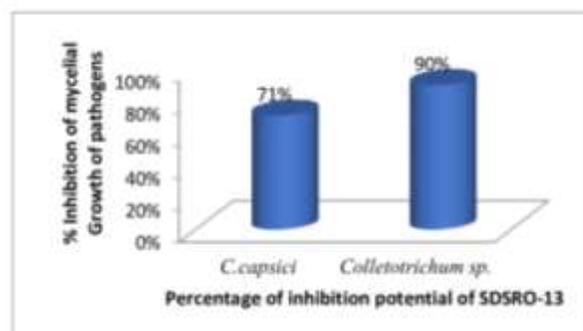


Fig. 7. Inhibition potential of SDSRO-13 against *Colletotrichum* strains

Thin layer chromatography

The fractionation of crude metabolite was carried out by TLC. Light pink coloured spot with Rf value of 0.68 was observed when treated with iodine vapours (Fig.8).

Antifungal activity of culture filtrate, solvent and solvent extract of SDSRO-13

The ethyl acetate extract of the SDSRO-13 metabolite was effective against the two test fungi of *Colletotrichum* pathogens of chilli

and zone of inhibition was between 2.5-19 mm (Fig. 8). Culture filtrate showed the highest zone of inhibition for *C. capsici* while for *Colletotrichum* sp. solvent extract showed the highest zone of inhibition (Fig. 9).

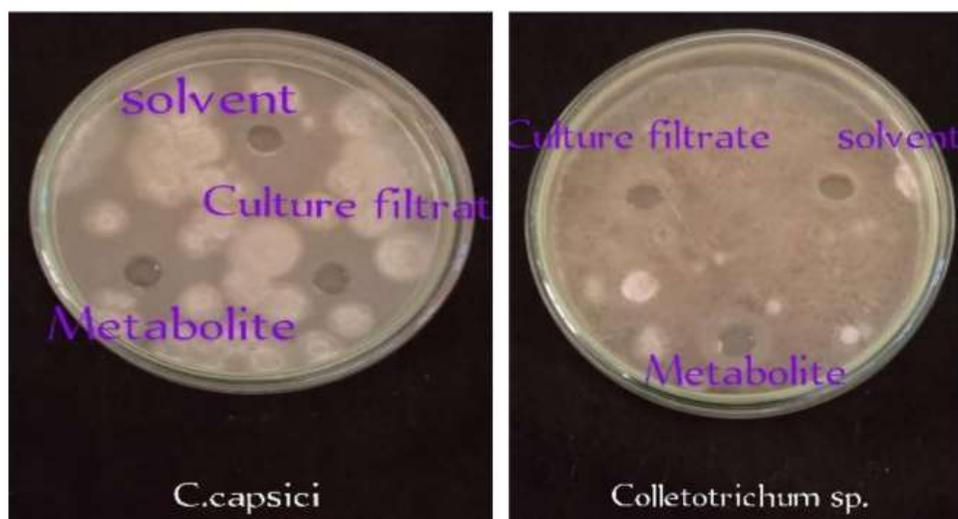


Fig. 8. Antifungal activity of culture filtrate, solvent and solvent extract of SDSRO-13

Pathogenicity test of SDSRO-13 against *Colletotrichum* strains of chilli

Anthracoze is one of the key problems that cause mature *C. annuum* fruits to suffer severe loss and decay (Rhavena, *et al.*, 2019). Pathogenicity of *C. capsici* and *Colletotrichum* sp. were observed for upto 21 days, after inoculation of the chilli fruit with the spore suspension of the pathogens. Inoculation of chilli fruits with the fungal spore suspension was done by making a small puncture on the fruit, first 3 days there was no any growth observed. On 5th day, the growth was initiated as white colored mycelium on the fruit. But in case of isolated *Colletotrichum* sp., slight mycelial growth was observed on 3rd day. The moist

chamber was maintained for the growth and establishment of the pathogen. On 21st day, the severity was about 70% by *C. capsici* and upto 90% in case *Colletotrichum* sp. *Colletotrichum* sp. was more virulent than *C. capsici*. When the fruits were treated with the isolate SDSRO-13 and two *Colletotrichum* strains no predominant growth was observed on the chilli fruit. The cell free suspension of the isolate SDSRO-13 was sprayed at four days of interval. Earlier studies also reported similar findings (Jeffrey *et al.*, 2015). The SDSRO-13 isolate was very potent against the two *Colletotrichum* strains. This actinomycete isolate was very promising which can be used in agricultural field to control anthracnose disease.

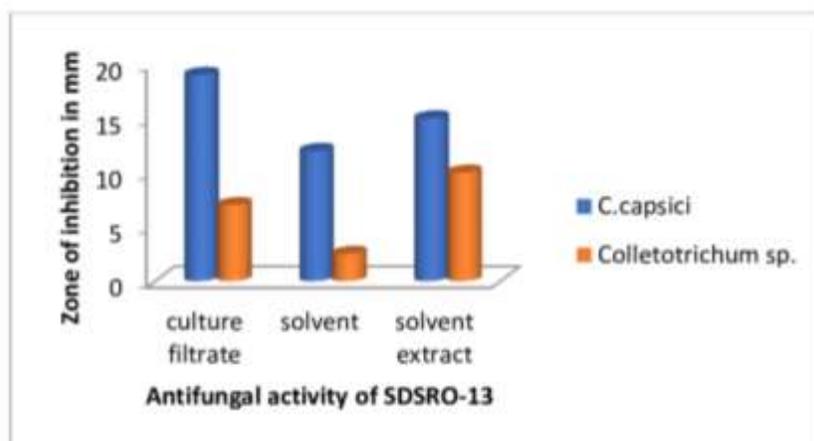


Fig. 9. Antifungal activity of culture filtrate, solvent and solvent extract of SDSRO-13

Similar results were obtained by Khucharoenphaisan *et al.* (2013) with *Streptomyces* sp. *S. malaysiensis* R58 culture filtrate. They reported that treatment with *S. malaysiensis* R58 reduced the anthracnose disease of chilli fruits significantly. This indicated that *S. malaysiensis* R58 can be used to control anthracnose disease as biological control.

Conclusion

Actinomycetes are well-known for their metabolites and secondary metabolism. The largest documented group of actinomycetes is *Streptomyces*. In the present work, 18 *Streptomyces* spp. were isolated from the maize rhizosphere soil. *In vitro* antifungal activity against *Colletotrichum* spp. was carried out by primary and secondary screening. Primary screening was carried out by cross streak method. Secondary screening was carried out by dual culture method by the potent isolate SDSRO-13. *In vivo* antifungal activity was carried out by inoculating the chilli fruits with the spore suspension of the test pathogens. Chilli fruits exposed to test pathogens and

SDSRO-13 showed no signs of anthracnose disease. Molecular studies lead to the identification of *Streptomyces* isolate SDSRO-13 as *Streptomyces rochie*. The isolated *Colletotrichum* sp. was identified as *Colletotrichum truncatum*. The *Streptomyces* isolate SDSRO-13 proved effective against two *Colletotrichum* strains, therefore it can eventually be utilized as an alternative to conventional fungicides in agricultural fields.

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References

- Altschul S F, Gish W, Miller W, Myers E W, Lipman D J 1990 Basic local alignment search tool. *J. Mol. Biol.* 215(3): 403-410.
- Augustine S K, Bahvsar S P & Kapadnis B P 2005 A non polyene antifungal antibiotic from *Streptomyces albidoflavus* PU23. *J. Biosci.* 30(2): 201-211.

- Darby A C, Chandler S M, Welburn, S C & Douglas A E 2005 Aphid-symbiotic bacteria cultured in insect cell lines. *Applied Environmental Microbiology*. 71(8): 4833-4839.
- Haritha R, Siva Kumar K, Jagan Mohan Y S & Ramana T 2010 Amylolytic and Proteolytic actinobacteria isolated from Marine Sediments of Bay of Bengal. *Int. J. Microbiol. Res.* 1(2): 37-44.
- Hassan O, Jeon J Y & Chang T 2018. Molecular and morphological characterization of *Colletotrichum* species in the *Colletotrichum gloeosporioides* complex associated with persimmon anthracnose in South Korea. *Plant Diseases*. 102(5): 1015-1024.
- Hyi J K, Eun J L, Sung H P, Hoi S L & Namhyun C. 2014 Biological Control of Anthracnose (*Colletotrichum gloeosporioides*) in Pepper and Cherry Tomato by *Streptomyces* sp. A1022. *J. Agric Sci.* 6(2): 1-10.
- Jeffrey L S H, Umi K M S, Nor A A R, Khozirah S & Halizah H 2015 *Streptomyces ambofaciens* S2 - A Potential Biological Control Agent for *Colletotrichum gloeosporioides* the Causal Agent for Anthracnose in Red Chilli Fruits. *Int. J. Plant Pathol. Microbiol.* 4: 1-7.
- Jisha M.S, Linu M.S & Sreekumar J 2018 Induction of systemic resistance in chilli (*Capsicum annuum* L.) by *Pseudomonas aeruginosa* against anthracnose pathogen *Colletotrichum capsici*. *J. Trop. Agric.* 56 (2): 153-166.
- Karlin S & Altschul S F 1990 Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. *Proceedings of the National Academy of Sciences. USA.* 87: 2264-2268.
- Khucharoenphaisan K, Sripairoj N, & Sinma K 2013 Isolation and identification of *actinomycetes* from termite's gut against human pathogen. *Asian Journal of Animal and Veterinary Advances.* 7: 68-73.
- Kunova A, Bonaldi M, Saracchi M, Pizzatti C & Chen X 2016 Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. *BMC Microbiology.* 16: 272. 1-11.
- Linu M S, Jisha M S & Jisha M S 2017 *In vitro* control of *Colletotrichum capsici* induced chilli anthracnose by fungicides and biocontrol agent. *International Journal of Applied and Pure Science and Agriculture.* 3(5): 1-7.
- Mesta S C & Onkarappa R 2017 Characterization and antimicrobial potential of marine actinomycetes from estuaries of Uttara Kannada district, Karnataka. *Journal of Drug Delivery & Therapeutics* 7(6): 27-37.
- Mills P R, Sreenivasaprasad S & Brown A E 1992 Detection and differentiation of *Colletotrichum gloeosporioides* isolates using PCR. *FEMS Microbiology Letter.* 98: 137-144.

- Mohammadipanah F & Wink J 2016 Actinobacteria from Arid and Desert Habitats: Diversity and Biological Activity. *Frontiers in Microbiology*. 6:1541. 1–10.
- Myers E W & Miller W 1988 Optimal alignments in linear space. *Computational Applied Biosciences*. 4(1): 1–11.
- Pakdeevaram P, Wasee S, Taylor P J & Mongkolporn O 2005 Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in capsicum. *Plant Breeding*. 124(2): 206–208.
- Pavithra S, Akila R, Rajinimala N, Gangai Selvi R & Kannan R 2019 Plant extraction mediated mitigation of chilli fruit rot caused by *Colletotrichum* spp. *Journal of Pharmacognosy and Phytochemistry* 8(4): 2879–2883.
- Petriccione M, Zampella L, Mastrobuoni F & Scortichini M 2017 Occurrence of copper-resistant *Pseudomonas syringae* pv. *syringae* strains isolated from rain and kiwifruit orchards also infected by *P. s.* pv. *actinidiae*. *European Journal of Plant Pathology*. 149: 953–968.
- Rahman A U & Humainy A A 2011 Taifcidin 1 and Taifcidin 2, two antimicrobial agents isolated from the fermentation broth of *Streptomyces roseodistaticus* TA15 and *Streptomyces lavendofoliae* TA17. *Research journal of Microbiology*. 6(4): 328–342.
- Reddy M K, Srivastava A, Kumar S R, Kumar R, Chawda N A, Ebert A W & Vishwakarma M 2014 Chilli (*Capsicum annuum* L.) breeding in india. *SABRAO Journal of Breeding and Genetics*. 46 (2): 160–173.
- Rhavana G L, Maria I S F & Marcos A S 2019 *Streptomyces griseocarneus* R132 controls phytopathogens and promotes growth of pepper (*Capsicum annuum*). 138: 1–9
- Rodríguez M, Torres M, Blanco L, Béjar V, Sampedro I & Llamas I 2020 Plant growth-promoting activity and quorum quenching-mediated biocontrol of bacterial phytopathogens by *Pseudomonas segetis* strain P6. *Scientific Reports*. 10: 1–12.
- Sabaratnam S & Traquair J A 2015 Mechanism of antagonism by *Streptomyces griseocarneus* (strain Di944) against fungal pathogens of greenhouse-grown tomato transplants. *Canadian J. Plant Path.* 37:197–211.
- Sahin N & Ugur A 2003 Investigation of the Antimicrobial activity of some *Streptomyces* isolates. *Turkish Journal of Biology*. 27: 79–84.
- Sandra Pacios M, Cristobal N, Aguilar G, Olga B, Alvarez-Perez, Raul R H, Mónica C G, Roberto A V, Juan A, Ascacio V, Mayela G S & Anna I 2021 Application of *Streptomyces* Antimicrobial Compounds for the Control of Phytopathogens. *Frontiers in Sustainable Food Systems*. 5: 1–13.
- Saputri E K, Amalia D & Wahyuni W S 2021 *Streptomyces* sp. has different efficacy to control two different pathogens. *J. earth environ. sci.*759: 1–6.

- Sharma M & Manhas R K 2020 Purification and characterization of salvianolic acid B from *Streptomyces* sp. M4 possessing antifungal activity against fungal phytopathogens. Microbiological research. 237: 1–14
- Shrivastava P, Kumar. R & Yandigeri M S 2017 *In vitro* biocontrol activity of halotolerant *Streptomyces aureofaciens* K20: A potent antagonist against *Macrophomina phaseolina* (Tassi) Goid. Saudi J. Bio Sci. 24: 192–199.
- Sun D, Liu C, Zhu J & Liu W 2017 Connecting metabolic path-ways: sigma factors in *Streptomyces* spp. Frontiers in Microbiology. 8:2546. 1–7.
- Tatsuya O, Minori M, Tetsuya M, Masahiro O, Shinhiro K & Takashi A 2018 *In vitro* and *In vivo* Assay for Assessment of Biological Control of *Streptomyces* sp KT. J. Plant Stud. 7(1): 1–14.
- Vashistha A & Chaudhary A 2019 Effects of biocontrol agents and heavy metals in controlling soilborne phytopathogens. Int. J. Herb. Med. 7: 41–45.
- Veerendra G, Arvinder K, Pramod K F & Himanshu S G 2017 Comparative studies on isolation of *Colletotrichum capsici* causing anthracnose disease of chilli. Int. J. Chem. Stud. 5(6): 744–747.
- Waksman S A & Henrici A T 1943 The nomenclature and classification of the actinomycetes. J. Bacteriol 46: 337–341.
- Zee K Y & Vu T A 2018 Effect of *Trichoderma* sp. on Anthracnose Disease of Stored Chilli. Borneo J. Resour. Sci. Technol. 8(2): 90–10.
- Zhuang X, Congting G, Chenghui P, Zhiyan W, Junwei Z, Yue S & Chongxi L 2020 Characterization of a Novel Endophytic Actinomycete, *Streptomyces physcomitrii* sp. nov. and Its Biocontrol. Potential Against *Ralstonia solanacearum* on Tomato. Microorganisms. 8: 1–12.