RESEARCH ARTICLE

Enhancing Crop Production using *Streptomyces* sp. *NCIM 5814* for Simultaneous Biocontrol of *Erwinia carotovora, Xanthomanas campestris* and *Xanthomonas axonopodis*

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Abstract

Bacterial infections caused by phytopathogens like *Erwinia carotovora* (*Ec*), *Xanthomonas campestris* (*Xc*) and *Xanthomonas axonopodis* (*Xa*) deteriorate important crops like rice, tomato, Pomegranate, chilli, citrus crops and other vegetables causing loss in food produce and economy. The rapid Propagation of these phytopathogens make it difficult to control their spread. The chemicals in form of pesticides and insecticides worsen the situation and cause negative impact on soil productivity, environment and human health. To overcome this, microbial agents like actinobacteria are being employed as source of sustainable farming. In the present study 76 actinobacteria were isolated and tested for their ability to control *Ec*, *Xc* and *Xa*. The primary screening revealed 12 potential isolates that could counter all three pathogens. Isolate P2 and T2 exhibited the highest inhibition zone against all three pathogens in secondary screening. The 16S rRNA study revealed that both actinobacterial species were closely related to *Streptomyces erumpens*. *In-vivo* treatment of the antibiotic produced by actinobacterial isolate P2 was carried out on *Solanum lycopersicum* and *Capsicum frutscens* in field conditions. The plants infected with phytopathogen were treated using crude antibiotic extract (CAE) that protected the plant from disease and also improved its growth. It was observed that the shoot length of treated plants was 33-37% higher in *Capsicum frutscens*. The CAE enhanced the plant weight by 69 to 135% in *Solanum lycopersicum* and 22 to 114% in *Capsicum frutscens*. *Capsicum frutscens* plants were found to have enhanced root weight in the range of 57 to 73%. The CAE also promoted branching and fruiting in both chilli and tomato plants, suggesting its possible utilization as a biofertilizer for sustainable agriculture.

Keywords: Actinobacteria; *Erwinia carotovora*; *Xanthomonas campestris; Xanthomonas axonopodis Solanum lycopersicum; Capsicum frutscens*.

Highlights:

- Actinobacteria are prolific producers of antibacterials.
- *Streptomyces* sp. *NCIM 5814* has the potential to control three major phytopathogens of food crops namely *Erwinia carotovora, Xanthomonas campestris* and *Xanthomonas axonopodis*.
- *Streptomyces* sp. *NCIM 5814* exhibited significant biocontrol effect on the infected *Solanum lycopersicum* and *Capsicum frutscens* in field conditions.
- *In-vivo* studies revealed considerable plant growth promotion prospective of the selected actinobacterial isolate.
- Plant weight, plant height, root length and root weight were significantly increased by treating with *Streptomyces* sp. *NCIM 5814.*

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Introduction

Sustainable development demands the adoption of environment-friendly agricultural practices to enhance the productivity of food crops. There is an urgent requirement for increasing the agricultural produce due to increase in population. Since the intervention of green revolution, the rising demand of food to feed the growing population has been fulfilled. To enhance the agricultural produce, uninterrupted use of chemicals in the form of fertilizers and pesticides is done, which is leading to dreadful diseases among humans. A biological approach is required to enhance the production of crops and also to counter the phytopathogenic organisms that deteriorate the agricultural produce.

Actinobacteria are extensively explored for searching antimicrobials against plant and human pathogens. They are a diversely distributed group of microorganisms that are found in almost all ecological niches (Dede *et al.*, 2020). These organisms are well-known producers of bioactive compounds (Krishanti *et al.*, 2018). *Streptomyces* are the most common genera of actinobacteria that persist in nature and play a major role

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in nutrient recycling and enhancing the productivity of soil. Different *Streptomyces* sp., like *S. olivaceoviridis*, *S. aureofaciens*, *S. avermitilis,* and *S. lividans,* have been reported to have antagonistic activity against various crop pathogens (Ling *et al.*, 2020). These actinobacteria have the potential to control crop plant disease and also enhance their growth.

Each year large amount of crop produce is lost due to phytopathogenic infections. These pathogens are responsible for an annual loss of 40 billion dollars worldwide (Pandit *et al.*, 2022). The disease caused by plant pathogens, including bacteria, fungi, viruses and other microbes, pose a big problem for agriculture and demand an urgent need for antimicrobial agents that can prevent microbial infections in agricultural crops (Rajaram *et al.*, 2020). In this study, we have attempted to control phytopathogens such as *Erwinia carotovora (Ec), Xanthomonas campestris (Xc),* and *Xanthomonas axonopodis (Xa).* which intensively harm horticulture crops. *Erwinia carotovora* is a major pathogen of tomato, chilli, pomegranate, capsicum, potato and other vegetables that causes damage of crops by producing pectic enzymes, cellulase, hemicellulase, protease and arabanase. *Erwinia carotovora* is responsible for reducing agricultural produce by causing soft rot disease in the crop plant (Dinkou et al., 2021). This phytopathogen is capable of infecting the crops in the field and also during storage. The loss of yield due to this bacterium is estimated to be up to 60% (Sulaiman *et al*., 2020). The crops infected by this Gram-negative bacterium cause plant tissues to turn slimy and foul-smelling (Akbar *et al*., 2015). Similarly, another phytopathogenic bacterium, *Xanthomonas campestris* infects potatoes, tomatoes, chilli and other cruciferous crops, causing huge damage to the agricultural produce of these vegetables. *Xanthomonas campestris* is a gram-negative bacilli with a single polar flagellum (Holtappels *et al*., 2022). It causes black rot disease, which is one of the most damaging diseases in crop fields and results in significant economic loss to the farmer. This phytopathogen causes the blackening of vascular tissues and foliar lesions, rendering the crops unfit for consumption. *X. campestris* is known to grow epiphytically and is capable of infecting the plant through hydathodes or wounds. It can spread through contaminated seeds, irrigation water, contaminated soil and insects (Liu *et al.*, 2016). A significant loss of 50 to 70% in the yield of cruciferous crops like cabbage and cauliflower has been reported due to *X. campestris* infection (Ramachandra et al., 2024). *Xanthomonas axonopodis* is responsible for causing bacterial blight in pomegranates. This phytopathogen is also known to cause blight and spot disease in soybeans, onions, tomatoes, rice and, citrus crops etc. This bacterium is capable of infecting all the stages of the plant. Its primary infection comes from infected seeds, plant debris or seedlings. The phytopathogen can penetrate through hydathodes and infect the whole plant. The disease progresses rapidly during humid conditions, causing the leaves to collapse and eventually killing the plant (Nga *et al*., 2021). *X. axonopodis* can lead upto 100% of the yield loss when the environmental conditions are conducive and its infection has been reported from almost all the pomegranate growing regions of India (Kumar *et al.*, 2020). This phytopathogen has even developed resistance to streptomycin and are now more difficult to remove from the agricultural field once their infection starts spreading (Behlau *et al*., 2012). Unfortunately, to date, insufficient attention has been paid to find appropriate biological control measures against *X. campestris*, and *X. axonopodis* which are among the most economically harmful pathogens (Macionienė *et al*., 2022).

Currently, farmers are controlling these phytopathogens using chemicals in excessive amounts which is severely impacting human health. The present investigation aims at finding an effective antibacterial agent against phytopathogenic bacteria *Erwinia carotovora*, *Xanthomonas campestris* and *Xanthomonas axonopodis* to reduce the annual loss of crop and to enhance the agricultural produce

Materials and Methods

All the chemicals used in the present study were purchased from Hi-Media, Mumbai (India). The phytopathogenic bacteria *Xanthomonas campestris, Xanthomonas axonopodis* and *Erwinia carotovora* were procured from Indian Agricultural Research Institute, New Delhi.

Isolation of actinobacteria

Actinobacteria were isolated from soil samples collected from different regions of Madhya Pradesh, India. Selective isolation of actinobacteria was done by adding 20 µg/mL of penicillin as an antibacterial and 20 µg/mL of amphotericin B as an antifungal agent in Bennett's and Actinomycete isolation agar. The isolated cultures were preserved on Bennett's agar slants (Singh *et al.,* 2020).

Primary screening for antibiotic-producing actinobacteria

Primary screening for antibiotic producing isolates was done by plate assay method. The cultures were spot inoculated on a modified Soybean meal medium (Rao *et al.*, 2015) and incubated at 30°C for 4 days. After the development of actinobacterial growth on these plates, it was treated with chloroform fumes for 30 minutes. The phytopathogen was inoculated in molten nutrient agar and poured on the actinobacterial growth. These plates were then incubated at 30°C for 24h to observe zone of inhibition (Medo *et al.*, 2019). The presence of zone of inhibition was checked on the upper layer of agar containing test organism for determining the production of antibiotics by actinobacteria growing on the lower layer.

Secondary screening for antibiotic producing actinobacteria

Fermentative Production of Antibiotic

Secondary screening for the selected actinobacterial isolates was performed by submerged fermentation technology. The inoculum for the production of antibiotics by selected isolates was developed by spot inoculating the isolates on Soybean meal agar medium. The inoculum was used after growing for 96h on the inoculum development medium (Soybean meal agar).

Soybean meal broth medium was prepared and 20 mL of this was dispensed in 100ml conical flasks. Inoculum disc of 8mm diameter was introduced in the production medium. The submerged fermentative production was carried out at 30°C for 96 hours in a CIS-24 BL orbital shaker.

Preparation of Crude Antibiotic Extract

The antibiotic produced was extracted by the addition of methanol in the ratio 1:1. The extraction process was carried out in a rotatory shaker at 37°C for 2 hours. The fermented broth mixed with methanol was harvested after 2 hours and centrifuged at 5000×g for 10 mins. The supernatant was used as crude antibiotic extract for performing an antibacterial assay against *X. axonopodis, X. campestris* and *E. carotovora*.

Antiphytopathogenic Bioassay

Antiphytopathogenic activity was determined by the cup-diffusion method. The inoculum of test pathogen *Erwinia carotovora* (*Ec*) was developed in nutrient broth at 37°C for 24 hours and *Xanthomonas campestris* (*Xc*), *Xanthomonas axonopodis* (*Xa*) was developed in peptone sucrose broth at 30°C for 48 hours. Test organism (0.1ml) suspension of optical density corresponding to 0.5 Mac Farland standard was inoculated in nutrient broth for *Ec* and molten peptone sucrose medium for *Xc* and *Xa* and poured in sterile perti dishes. About 80 microlitres of crude antibiotic extract was dispensed in 8 mm wells in the plates. These plates were incubated at 30°C and the results were observed after 48 hours (Ravi and Kannabiran, 2018). The promising isolates were chosen for further study.

Identification of actinobacteria using 16S rRNA Sequencing

The secondary screening revealed actinobacterial isolate P2 and T2 to be the best performers among 76 actinobacterial isolates. Thus, these two cultures were picked up for identification process by 16S rRNA gene sequencing approach.

The chromosomal DNA of both actinobacterial isolates P2 and T2 was isolated using the DNA isolation spin column kit (HiMedia). The 16S rRNA was amplified using 27F (5′-GAGTTTGATCMTGGCTCAG-3′) as the forward primer and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) as the reverse primer. The PCR product was purified using Exonuclease 1-Shrimp Alkaline phosphatase (Exo-Sap), and then the amplicons were sequenced through Sanger's method using a ABI 3500×L genetic analyzer (Life Technologies, USA). This process of identification of actinobacterial species was carried out at National Collection of Industrial Microorganisms (NCIM), NCL-CSIR, Pune, India.

The sequence obtained was matched with the GenBank database using BLASTN algorithm to reveal the closest match of 16S rRNA from the known species. Phylogenetic tree was constructed using MEGA X software by aligning the sequence of our isolate with its closely related individuals picked up from BLAST result.

Effect of actinobacterial extract on *Solanum lycopersicum* and *capsicum frutscens* plants

The application studies were conducted in order to check the biocontrol effect of *Streptomyces* sp. *NCIM 5814* against the infection of phytopathogens *E. carotovora, X. axonopodis* and *X. campestris* in tomato and chilli plant. *In-vivo* treatment of the antibiotic produced by *Streptomyces* sp. *NCIM 5814* was done on *Solanum lycopersicum* and *Capsicum frutscens* in field conditions.

The experiment was conducted in the agricultural field of Jana Pav (22.45°N, 75.68°E), which is situated in Indore district, Madhya Pradesh, India. The annual average temperature here is 75°F and rainfall is usually 96mm. Plants in the experiment were spread over a plot area of 10000 square feet.

Experiment Design

The design of experiment included 3 control sets and 3 test sets (each having 3 plants) as mentioned in Table 1. Control 1 were untreated plants, Control 2 were treated with supernatant of *Streptomyces* sp. *NCIM 5814* fermented broth, Control 3 Ec were infected with *E. carotovora,* Control 3 Xc were infected with *X. campestris* and Control 3 Xa were infected with *X. axonopodis* phytopathogen. Test 1 was first infected with phytopathogen *E. carotovora* and then treated with Crude Antibiotic Extract (CAE) which is basically the supernatant of *Streptomyces* sp. *NCIM 5814* fermented broth in order to check biocontrol by the selected isolate. Test 2 was first infected with phytopathogen *X. campestris* and then treated with CAE and Test 3 was first infected with phytopathogen *X. axonopodis* and then treated with CAE. The whole experiment was conducted separately in triplicates for all the 3 pathogens i.e. *E. carotovora, X. campestris* and *X. axonopodis* in chilli and tomato plants. Plantlets were of an average similar height, around 15 cms. and age, 15 to 20 days.

Phytopathogens were grown in the respective suitable medium at optimum conditions. All the test plants were rubbed with sandpaper to create abrasion on the tissue to facilitate

Table 1: The experimental design of the study

Title	*Capsicum frutscens*	*Solanum lycopersicum*
Control 1	Planted in untreated condition	Planted in untreated condition
Control 2	Plant treated with CAE	Plant treated with CAE
Control 3 *Ec*	Plant infected with *Erwinia carotovora*	Plant infected with *Erwinia carotovora*
Test 1	Plant infected with *Erwinia carotovora* and treated with CAE	Plant infected with *Erwinia carotovora* and treated with CAE
Control 3 *Xc*	Plant infected with *Xanthomonas campestris*	Plant infected with *Xanthomonas campestris*
Test 2	Plant infected with *Xanthomonas campestris* and treated with CAE	Plant infected with *Xanthomonas campestris* and treated with CAE
Control 3 *Xa*	Plant infected with *Xanthomonas axonopodis*	Plant infected with *Xanthomonas axonopodis*
Test 3	Plant infected with *Xanthomonas axonopodis* and treated with CAE	Plant infected with *Xanthomonas axonopodis* and treated with CAE

infection. The plants were then infected by spraying pathogens on the whole plant. The plants were then observed daily.

Antibiotic production was done by submerged fermentation using *Streptomyces* sp. *NCIM 5814*. On the development of symptoms of infection on the leaves, all the Test plants were treated with crude antibiotic extract of *Streptomyces* sp. *NCIM 5814* by spraying on the whole plant. Control 2 plants were also sprayed with CAE to observe the effect of the metabolites present in them on the uninfected plant. CAE was sprayed at an interval of 15 days for 2 times. The experiment was conducted for 3 months, and the plants were harvested on the 90th day. The response of plants was analyzed by measuring various parameters such as plant fresh weight, total plant length, shoot length, root length, root weight, no. of branches, number of leaves, number of fruits, and number of secondary roots.

Statistical analysis

All data of physiological parameters in different treatments were tested for significance using one-way ANOVA. The treatment means were compared using the least significant difference at $P < 0.05$.

Results and Discussion

Isolation of actionobacteria

Actinobacteria have been reported to be present ubiquitously throughout the soil and aquatic niches (Viswanathan and Jeyanthi 2017). These microbes are considered valuable as they can produce numerous enzymes for mineralization and diverse secondary metabolites (Dewi *et al*., 2015). In the present study, 76 actinobacterial cultures were isolated from the soil samples collected from different areas of Bhopal, Mandideep and Pachmarhi in M.P. The array of cultures was diverse in terms of cultural characters.

Most of our isolates exhibited white-colored aerial spore mass, which turns gray on aging. According to Bergey's Manual, such cultures predominantly belong to the group *Streptomyces*. Ten out of 76 isolates exhibited Streptomyces-like colonies. A few cultures sporulated scantily. The isolates were differentiated based on certain characteristic features like their colony morphology, colour and arrangement of aerial spore, reverse colony coloration and pigmentation, as described in the International Streptomyces Project and Bergey's Manual of Systematic Bacteriology (Shirling and Gottlieb 1966). Xue *et al.,* (2013) reported morphologically diverse 712 actinobacterial isolates which were isolated from the different crop fields. Similarly, Ouhdouch *et al.,* (2001) isolated 320 actinobacteria from different Moroccan habitats. Actinobacteria are predominant among microbial populations and are also potential candidates that can serve as biocontrol agents (Barka *et al*., 2016).

Primary screening for antibiotic producing actinobacteria

All the 76 isolated actinobacteria were primarily screened for the production of antibiotic against the three bacterial pathogens *viz., E. carotovora, X. campestris* and *X. axonopodis*. Among 76 actinobacterial isolates, 24 (31.5%) cultures were found to exhibit inhibitory activity against the tested phytopathogens. Twelve out of 24 cultures were commonly inhibiting all the three phytopathogens. Top 5 cultures exhibiting good antagonistic activity against all the phytopathogens were picked up for further study.

Twelve (15.7%) were found to inhibit the growth of *E. carotovora* to a considerable extent. P2 created a zone of 45mm, T2 43.6 mm, EL2 42.3 mm and EL1 38.6 mm against *E. carotovora*. Soft rot disease of potatoes caused by *E. carotovora* is one of the widespread bacterial infection causing huge losses in the yield of potato. This is also one of the few diseases that can spread extensively in potato storage area, causing significant losses during the storage period (Helias *et al*., 2000). Karkouri *et al.,* (2010) found 13 actinobacterial isolates that were capable of inhibiting *E. chrysanthemi* and *E. carotovora* and developed an inhibition halo ranging from 5-32mm against them. Shalem and El-Shafea (2018), reported inhibition zones of *Streptomyces* sp. against two *E. carotovora* subsp. *carotovora* (Ecc1 and Ecc2) to

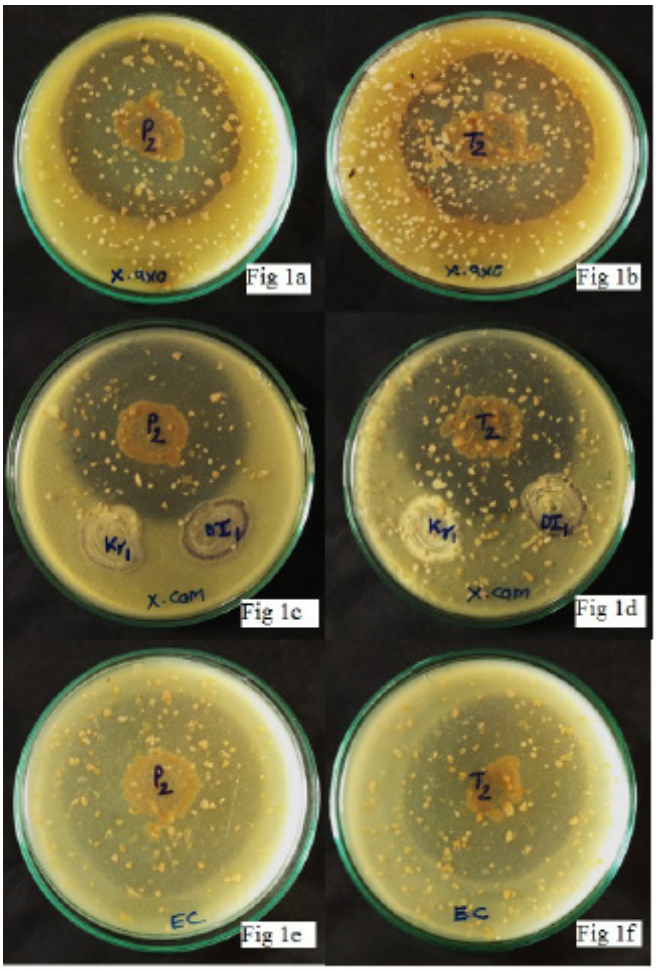

Fig. 1: Actinobacterial isolate P2 and T2 exhiting zone of inhibition against phytopathogens. Fig. 1a Isolate P2 inhibiting *X. axonopodis*. Fig. 1b Isolate T2 inhibiting *X. axonopodis*. Fig. 1c Isolate P2 exhibiting inhibition against *X. campestris*. Fig. 1d Isolate T2 inhibiting growth of *X. campestris*. Fig. 1e Isolate P2 inhibiting *Erwinia carotovora*. Fig. 1f Isolate T2 inhibiting *Erwinia carotovora*.

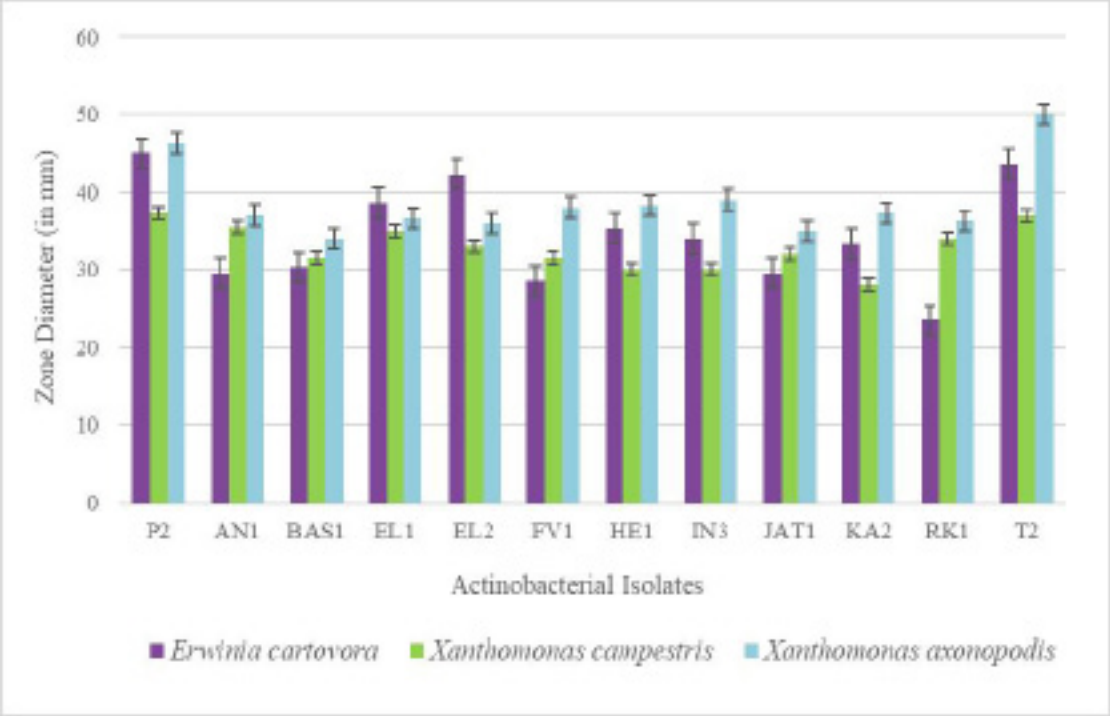

Fig. 2: Primary screening of actinobacterial isolates.

be 37 and 40mm. Our reserve was reasonably efficient against this phytopathogen,

Twenty (26.3%) among 76 isolates exhibited an inhibitory effect by seed plate technique against *X. campestris*. The inhibition found was quite high to the extent of 37.3mm of zone diameter produced by the isolate P2. Isolate T2 also exhibited nearly a similar result. Kang *et al.*, (2009) studied 316 *Streptomyces* sp. and only two were capable of inhibiting *X. Campestris* suggesting that biological control of this phytopathogen can be challenging Valan *et al.,* (2012) isolated 367 actinomycetes from Western Ghats of Tamil Nadu and 62 isolates (16.9 %) could inhibit *Xanthomonas* sp. Mingma *et al.,* (2013) isolated 312 actinobacteria from different niches and observed the highest inhibition ratio of 3.79. De Oliveira *et al.,* (2010) also utilized actinobacteria for the biocontrol of *Xanthomonas campestris* and reported that out of 70 isolates only 9% were capable of inhibiting this phytopathogen (Fig.1).

Twenty one (27.6%) among 76 isolates could inhibit the growth of *X. axonopodis* with inhibition zone diameters ranging from 50 to 19 mm. Our isolates P2 and T2 were highly effective against *X. axonopodis* producing 50 and 46.3 mm zone diameter, respectively. Besides these two isolates EL2, HE1, LG1, K1, KA2 and EL1 also very significantly inhibited all the phytopathogens. Results of primary screening are given in Fig. 2. Chavan *et al.,* (2016) reported 11 actinobacteria strains out of the 40 strains to be highly effective against *X. axonopodis*. *Streptomyces violaceusniger* strain A5 which was isolated from chitin-rich decomposed snake skin, showed strong inhibitory activity against *Xanthomonas axonopodis*, the causative agent of blight disease in pomegranate (Chavan *et al.*, 2016). Ma *et al.,* (2022) isolated 140 actinobacteria from soil among which isolate St-79 exhibited antagonistic effect against *X. axonopodis* and other phytopathogens.

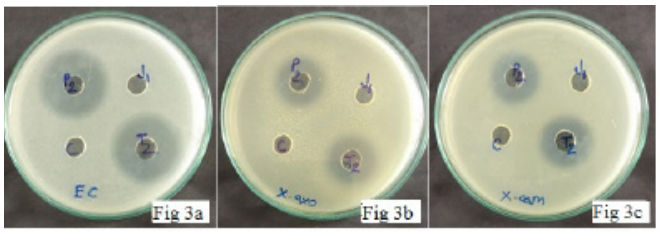

Fig. 3: Secondary screening of selected actinobacterial isolatate. Fig. 3a Isolate P2 and T2 inhibiting growth of *Erwinia carotovora*. Fig. 3b Isolate P2 and T2 inhibiting growth of *Xanthomonas axonopodis*. Fig. 3c Isolate P2 and T2 inhibiting growth of *Xanthomonas campestris*.

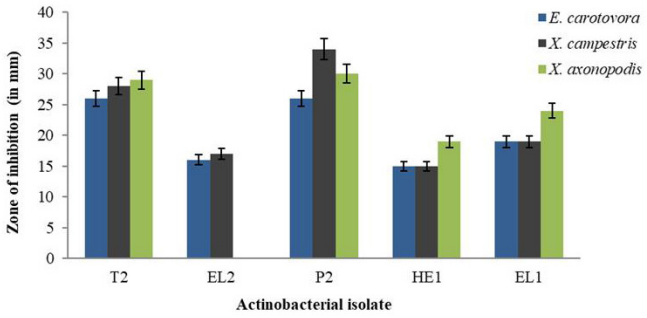

Fig. 4: Secondary screening of top five potential actinobacterial isolates.

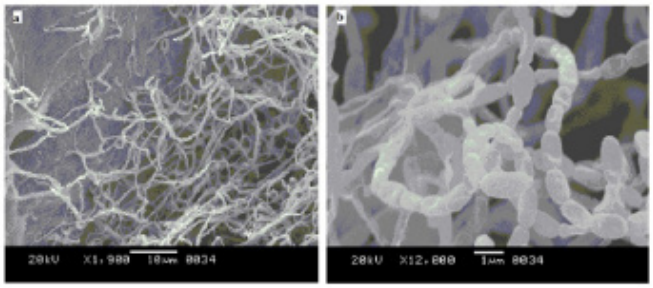

Fig. 5: (a) SEM image of actinobacterial isolate P2 at 1900X magnification (b) SEM image of actinobacterial isolate P2 at 15000X magnification

Secondary screening for antibiotic producing actinobacteria

Actinobacterial isolates selected by primary screening were subjected to secondary screening to test their antagonistic activity against phytopathogens. Antibiotic production with top twelve actinobacterial isolates was carried out by submerged fermentation technology and the antagonistic activity was assessed by agar well diffusion method. Antibiotic production was checked against all the three phytopathogens i.e. *E. carotovora*, *X. campestris* and *X. axonopodis*. Isolate P2 and T2 inhibited *Eriwinia carotovora* and *X. axonopodis* to the highest extent, whereas isolate IN3 along with P2 and T2 exhibited efficient antagonistic activity against *X. campestris*. Isolate P2 and T2 created a zone diameter of 34 mm, giving similar results against *E. carotovora*. We observed very good results against *X. campestris* also with isolate P2 creating an inhibition zone of 40mm diameter followed by 37mm by IN3 and 34mm by P2. *X. axonopodis* was inhibited maximum by P2 which created a zone diameter of 30mm followed by 29mm by T2. The secondary screening yielded two highly efficient actinobacterial cultures named P2 and T2 (Fig. 3 and Fig. 4)

Although actinobacteria are very well known for the production of antibacterials, we found 24 (31.5%) isolates inhibiting the growth of our test pathogens. As already reported by researchers Actinobacteria possess multiple biosynthetic gene clusters responsible for producing bioactive compounds, but many of the strains do not produce these compounds by routine fermentation processes. There are reports of enabling such strains to express the cryptic biosynthetic gene clusters by co-cultivation, elicitation, metabolic engineering, or some other relevant methods. Isolates other than these 24 cultures might have some cryptic gene clusters or may require some other medium components and physiological parameters for the expression of the genes. Our high-performing isolates also exhibited plant growth-promoting activity. Passari *et al.* (2016) amplified the genes iaaM and acdS in *Streptomyces* sp. DBT204, which is responsible for IAA, kinetin and antibiotic production. This isolate exhibited plant growth-promoting traits in both chilli and tomato plants. Our results also indicated huge variability among the antibacterials produced by our collection of isolates as different isolates inhibited different pathogens to varying extents. This accounts for the diverse genes possessed by the actinobacterial group of organisms, which are responsible for producing different bioactive compounds. Some isolates inhibiting all the phytopathogens support the gene synteny observed by the researchers in the actinobacterial genome.

Identification of actinobacteria

Morphological Analysis

Selected isolates grew as leathery colonies on Bennett's agar medium with scanty sporulation. The phase contrast microscope revealed a network of substrate hyphae above which aerial hyphae were observed branching into spore chains arranged in a spiral pattern. SEM images of the selected isolate are depicted in Fig. 5.

Molecular Characterisation

The potential actinobacterial cultures P2 and T2 were identified using 16S rRNA sequencing. The sequence of isolate P2 received after 16S rRNA sequencing was 1419bp long, and that of T2 was 1472bp long. 1413 bases out of 1419 bases of P2 matched with *Streptomyces erumpens strain 40941*. The query coverage was 100% with 99.58% identity. Our query sequence exhibited 5 insertions as it exhibited 5 gaps at positions 8, 24, 39, 723 and

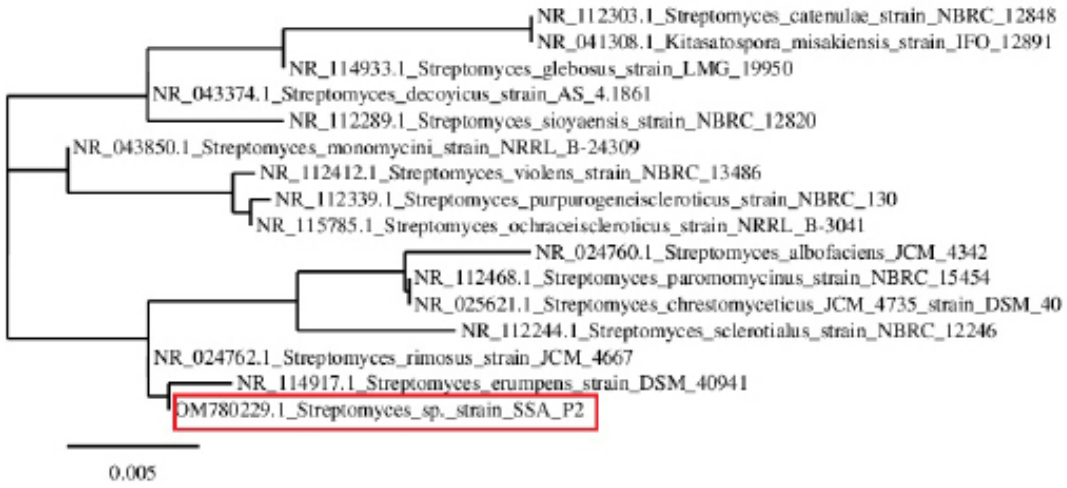

Fig. 6: Phylogenetic tree of isolate P2.

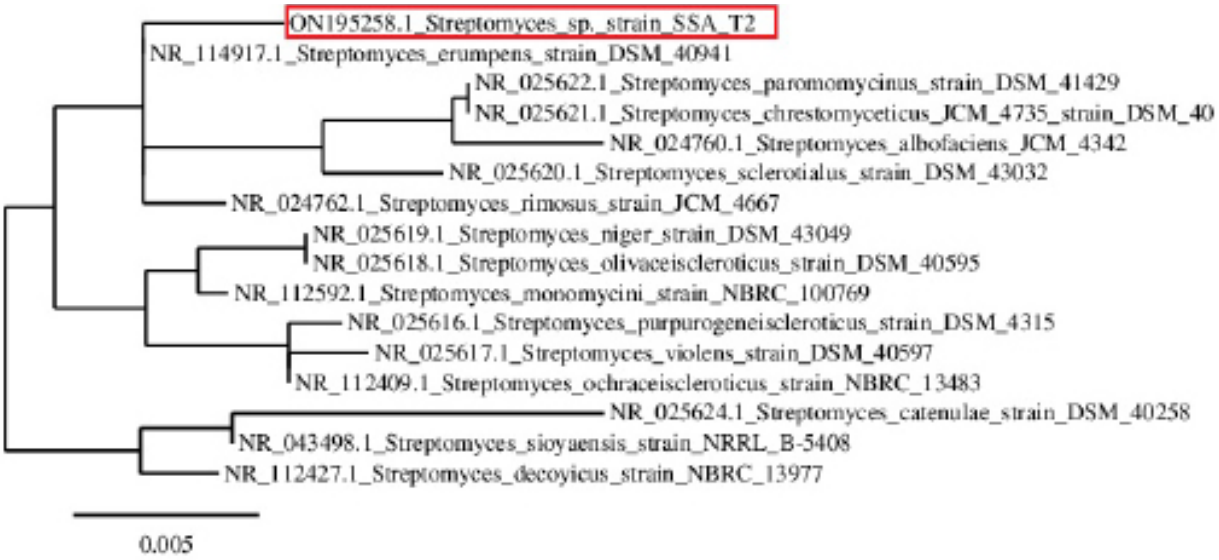

Fig. 7: Phylogenetic tree of isolate T2

1382. A mismatch was observed at position 1075 where query sequence had thymine and the response sequence had cytosine at this position.

Our query sequence of 1472 base pairs of isolate T2 matched at 1464 positions with the strain showing closest similarity, *Streptomyces erumpens strain 40941* implying 99.46% identity. 16S rRNA sequence of isolate T2 created 4 gaps in the closest similar sequence at positions 16, 31, 713 and 1409. A deletion was detected at position 717 and there were 3 mismatches at positions 716, 750 and 1468.

Both the isolates were identified as *Streptomyces* sp. and also were closely related to each other in spite of being isolated from samples picked up from different locations, as P2 was isolated from soil sample of the hilly region in Pachmari, Madhya Pradesh and T2 was isolated from a garden soil sample of densely populated city Bhopal, Madhya Pradesh. The 16S rRNA sequence of both the actinobacterial isolates was submitted to Genbank under the accession number OM780229 for P2 and ON195258 for T2. The phylogenetic tree was constructed using MEGA X program for both the isolates with 15 sequences that were closely related with isolate P2 and T2, respectively in the BLAST result (Fig. 6 and Fig. 7). *Streptomyces erumpens strain 40941* which was found to be closest to both the isolates P2 and T2 has been reported for the production of antibiotics (Vijayakumar *et al*., 2010) and amylase enzyme (Al-Agamy *et al*., 2021; Ratnakomala *et al*., 2020). BLAST results of both the isolates exhibited next closest organism as *Streptomyces rimosus,* which is also a well-known producer of antibiotics such as oxytetracycline (De Simeis and Serra, 2021).

Effect of actinobacterial extract on *Capsicum frutscens* and *Solanum lycopersicum*

In-vivo Studies

The experiment was conducted in a field containing black cotton soil which is free of any kind of chemical fertilizer or pesticide. The organic carbon content of the soil in which plants were grown was 0.37% with phosphate and nitrate nitrogen composition of 169 and 50 kg/hac, respectively.

In our study, the experiment design included Control 1 which was untreated, this plant was grown in the untreated soil. This control was planted in order to check the extent of fertility of the soil to support plant growth. Control 2 was treated with Actinobacterial culture supernatant by spraying the CAE (Crude Antibiotic Extract) of *Streptomyces* sp. *NCIM 5814*. This control was planted to check whether the crude extract has beneficial, deleterious or no effect on plant growth so that the results of test plants can be mapped. Control 3 was treated with the selected phytopathogen to observe the disease progression and effect on plant growth and productivity. Test plants were infected with the selected phytopathogens and after the development of disease symptoms, it was treated with CAE of *Streptomyces* sp. *NCIM 5814*. These plants were studied to check the biocontrol potential of our selected isolate and it's effect on plant productivity. The results clearly indicate the biocontrol potential of *Streptomyces* sp. *NCIM 5814* on the phytopathogens. *Streptomyces* are well-known producers of diverse bioactive compounds which makes them a suitable biocontrol agents. A variety of antibacterial agents like lankamycin and lankacidin C (Lu *et al*., 2018), aureomycin and avermectin (Cheng *et al*., 2018), actinorhodin (Cihak *et al*., 2017) are synthesized by *Streptomyces* sp.

Besides the biocontrol potential observed in the study, plant growth-promoting activity is also clearly indicated as nearly all the Control 2 plants which were treated with the CAE of *Streptomyces* sp. *NCIM 5814* grew healthy and strong with all the tested parameters to be better than Control 1 which was completely untreated. Actinobacteria have been reported to promote plant growth by assisting and providing various nutrients to it. They are known to increase the water intake and retention capacity of plants and provide soluble phosphorous, which is beneficial for plant growth (Schütze *et al*., 2014). Actinobacterial metabolites provide biological nutrients like N and P to the crop plant, which are preferred over agrochemicals, which cause serious damage to the environment

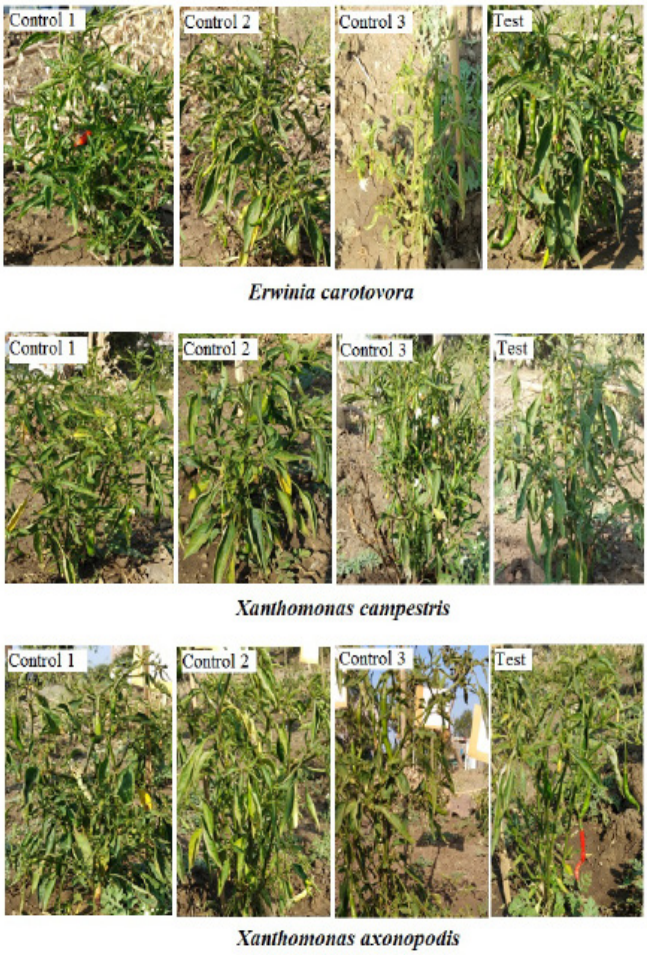

Fig. 8: Antagonistic effect of actinobacteria P2 against different phytopathogens in *Capsicum frutscens*.

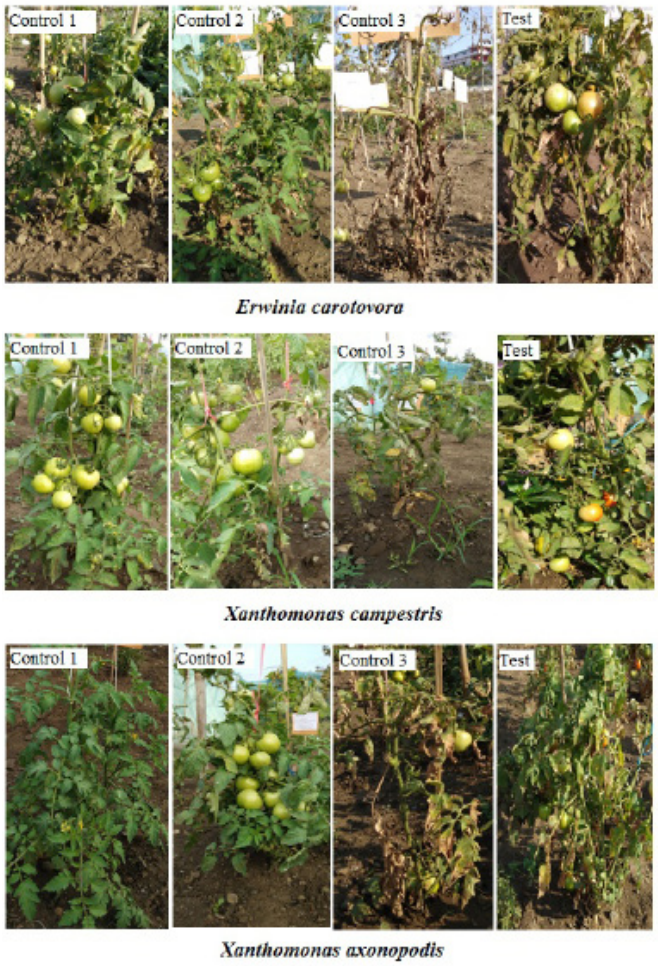

Fig. 9: Antagonistic effect of actinobacteria P2 against different phytopathogens in *Solanum lycopersicum*.

and are also expensive. They also influence the growth of plants by minimizing the deleterious effect of pathogenic microbes by producing antagonistic compounds (Tanvir *et al*., 2019).

Yellow spots of infection were observed on the Control 3 plants which were infected with the phytopathogens (Fig. 8 and Fig. 9), growth was stunted and plant fresh weight was reduced. The detailed results are given in Table 2 and Table 3.

Treatment of *Capsicum frutscens* and *Solanum lycopersicum* plants with CAE of *Streptomyces* sp. *NCIM 5814* exhibited profound effect on the test plants. Growth of the treated plants was much better than the infected plants. Actinobacteria play a critical role in promoting the growth of the plant through many mechanisms like PGP hormone production (Solá *et al*., 2019), plant growth regulation, siderophore production (Lee *et al*., 2012), plant protection against biotic stress, biocorrosion, and biodegradation/bioremediation (Limaye *et al*., 2017). Apart from promoting the growth of plant and protecting it against phytopathogen, actinobacteria also play a crucial role in organic acid production P solubilization (Farhat *et al*., 2015), K solubilization (Nafis *et al*., 2019), N fixation (Kucho *et al*., 2017), organic matter decomposition (Das *et al*., 2007) which overall improve the soil health. Omar *et al.,* (2022) observed that *Streptomyces* sp. could convert an insoluble phosphorous compound into its soluble form, allowing the plant to use it for assimilation. Actinobacteria are actively involved in nutrient management and thus affect soil fertility as nutrient enhancers (Jog *et al*., 2014).

All the three pathogens affected the *Solanum lycopersicum* plant fresh weight to a greater extent which was reduced in the range of 11 to 21% whereas chilli was less affected. Treatment of the infected plants with CAE increased the plant fresh weight by 22 to 114% in case of *Capsicum frutscens* and 69 to 135% in case of *Solanum lycopersicum*. This enhancement can be directly corelated with the Control 2 plant which was only treated with CAE and exhibited an increase of 76% in case of *Capsicum frutscens* and 146% in case of *Solanum lycopersicum*. These observations are indicating that the extract is not only protecting the plant from deleterious effects of infection but also providing plant growth promoting factors. Faddetta *et al.,* (2023) also demonstrated that the fermented broth from *Streptomyces* sp. AN090126 exhibited antimicrobial activity and was composed of different bioactive and plant growth promoting compounds like germicidin, siderophores like desferrioxamine E. The suppression of phytopathogenic effect and enhancent in

Table 2: Antagonistic effect of isolate P2 against phytopathogen *Erwinia carotovora* (*Ec*), *Xanthomonas campestris* (*Xc*), *Xanthomonas axonopodis* (*Xa*) in chilli plant. The values in the table represents Mean ± SD.

	Plant weight	*Plant length*	*Shoot length*	*Root length*	*Root weight*	*Secondary roots*	*Branches*	*Leaves*	*No. of fruits*
Control 1	39.79±1.67	45.84±5.23	23.79±2.14	22.05±3.77	3.04±0.33	52.33±15.04	27.67±3.51	79.67±8.62	14.33±4.51
Control 2	70.43±10.64	58.45±8.33	34.23±5.53	24.22±3.25	4.83±0.91	65.00±21.79	48.00±3.61	165.67±8.50	25.33±8.74
Ec infected Plant	34.63±4.91	39.92±3.37	20.27±2.11	19.64±2.16	2.69±0.41	51.00±10.15	22.33±3.51	65.33±20.43	9.00±5.57
Ec treated with P2	54.32±4.14	55.54±3.86	31.80±1.47	23.74±3.56	5.27±0.98	58.67±11.85	37.33±6.43	159.00±6.56	11.33±4.04
Xc infected plant	38.98±4.20	41.87±7.32	22.78±3.69	19.09±3.71	2.94±0.32	35.00±5.0	25.00±8.89	64.00±10.82	8.00±5.29
Xc treated with P2	48.93±3.14	65.34±5.93	32.68±2.46	32.66±3.90	4.78±1.98	60.33±4.51	45.33±6.51	155.33±6.43	17.33±1.15
Xa infected plant	50.16±12.48	45.60±1.35	22.98±2.82	22.63±2.55	3.57±0.61	40.67±4.04	23.00±8.54	101.67±11.93	12.67±4.73
Xa treated with P2	85.23±10.47	62.12±5.44	32.45±2.24	29.66±3.25	5.22±0.62	61.33±10.97	44.67±12.34	141.00±7.81	15.00±5.57

Table 3: Antagonistic effect of isolate P2 against phytopathogen *Erwinia carotovora* (*Ec*), *Xanthomonas campestris* (*Xc*), *Xanthomonas axonopodis* (*Xa*) in tomato plant. The values in the table represents Mean ± SD.

	Plant weight	*Plant length*	*Shoot length*	*Root length*	*Root weight*	*Secondary roots*	*Branches*	*Leaves*	*No. of fruits*
Control 1	106.95±12.82	89.33±8.33	57.22±4.59	33.14±3.99	4.99±0.20	40.67±5.13	46.67±4.62	146.67±10.69	20.67±3.06
Control 2	263.28±25.25	114.33±9.50	70.38±5.74	44.70±4.02	6.06±0.86	51.33±3.21	52.67±3.06	171.67±3.79	32.33±3.51
Ec infected Plant	85.62±20.22	72.00±14.42	48.15±9.60	24.94±4.83	4.19±1.41	31.67±0.58	38.00±8.00	118.67±17.62	11.67±6.51
Ec treated with P2	180.93±22.50	104.67±4.04	61.58±2.09	43.95±3.80	4.39±0.53	51.00±3.46	51.00±5.57	152.33±12.50	29.67±8.96
Xc infected plant	94.41±9.63	73.33±7.64	49.99±1.57	24.35±5.87	2.10±0.16	28.00±3.00	20.67±13.05	131.67±10.41	14.33±2.08
Xc treated with P2	251.52±12.71	111.00±8.72	73.11±5.58	38.64±3.62	5.85±0.89	51.67±3.51	50.00±10.15	170.33±1.53	28.67±8.08
Xa infected plant	84.13±16.28	56.33±11.37	38.30±9.76	18.86±1.45	2.20±1.67	22.67±6.43	29.00±7.55	142±13.89	16.33±2.08
Xa treated with P2	243.18±17.22	98.00±8.19	64.71±5.19	34.13±2.84	6.09±0.42	46.00±10.15	47.33±5.69	164±17.06	29.00±6.56

plant growth in our study also indicates the presence of similar compounds in the fermented broth. Different researchers have reported a variety of bioactives from actinobacteria that are capable of inhibiting phytopathogen. These interesting compounds includes antibiotics like prodiginines, actinorhodin (Vassallo *et al*., 2020) and tryptophan plant growth promoting (PGP) metabolites like IAA (Revelou *et al*., 2019).

Shoot length was affected maximum by *Erwinia carotovora* among the three pathogens tested in *Capsicum frutscens*, it was lesser by 14.7% as compared to the untreated and uninfected plant (Control 1) and *X. axonopodis* affected *Solanum lycopersicum* highest by reducing it's shoot length by 33%. This reduction was reversed when the plants were treated with CAE. The shoot length of treated plants were higher in the range of 33 to 37% in case of *Capsicum frutscens* whereas increase was lesser in case of *Solanum lycopersicum* but far better than the infected plants as detailed in the Fig. 9. Dias *et al.,* (2017) also observed significant increase in shoot length of *Solanum lycopersicum* plant when it was treated with different *Streptomyces* sp. Similarly, Devi *et al.,* (2022) also reported an enhancement of shoot length of *Solanum lycopersicum* by 78% after it was treated using *Streptomyces* sp. SP5. These findings clearly support that *Streptomyces* not only protect the plant from phytopathogenic infections but also enhances the plant growth.

Root length was more affected in *Solanum lycopersicum* as compared to *Capsicum frutscens*. *Erwinia carotovora* reduced the

length of root by 24.73%, *Xanthomonas campestris* reduced it by 26.6% and *Xanthomonas axonopodis* affected the root by 43% in *Solanum lycopersicum*, whereas *X. axonopodis* could not reduce the root length in *Capsicum frutscens* and *X. campestris* affected *Capsicum frutscens* by 13.45%. Treated plants did not show the suppression in the root length rather there was an increase of 48% in *Capsicum frutscens* test 2 plants and 32% in case of test 1 plants in *Solanum lycopersicum* which indicates that the CAE not only antagonized the infection but also served as bioinoculant. Kanini *et al.,* (2013) have isolated *Streptomyce rocheii* ACTA1551 from rhizosphere of *Pinus brutia* which efficiently protected tomato plant from phytopathogenic microbes and promoted its root and shoot growth. Niu *et al.,* (2022) also observed enhanced root length and root weight of 42.9 and 39.3% in *Panickum virgatum* when it was treated using *Streptomyces alfalfae* strain 11F. The bioactive compound Strevertenes A and B were reported to be produced by *Streptomyces psammoticus* KP1404 which were able to supress the wilt development in tomato plant, promoting its better growth (Kim *et al.*, 2011). Goudjal *et al.,* (2013) reported the presence of IAA in the culture filtrate of *Streptomyces* sp. PT2 promoted the root elongation and seed germination of the tomato plant.

There was a profound enhancing effect observed in the root weight and number of secondary roots in the test plants treated with fermented broth. Root weight was also supressed greatly by both *X. campestris* and *X. axonopodis* by 57.8 and 56% respectively in *Solanum lycopersicum*. This also influenced the reduction in the number of secondary roots. *Capsicum frutscens* was comparatively resistant to infections and did not exhibit considerable reduction in root weight. Treated *Capsicum frutscens* plants were found to have greatly enhanced root weight in the range of 57 to 73% and *Solanum lycopersicum* exhibited marginal increase but was much better than the infected plants as depicted in Fig. 9. Number of secondary roots were reduced to highest by 33.12% in *X. campestris* infected *Capsicum frutscens* and to 44% in *X. axonopodis* infected *Solanum lycopersicum*. The test plants were not affected by the pathogens, moreover, there was an increase of 15% in test 2 of *Capsicum frutscens* and 13% in test 3 of *Solanum lycopersicum*. Actinobacteria play a major role in shaping the root microbiome by modulating the composition of root and nutritional exchanges. Plant root exudates are the source of metabolic signals (such as strigolactones, flavonoids, and terpenoids), which participate in shaping the rhizosphere microbial communities. Streptomycetes colonize the root tissues by entering the root from the rhizosphere. IAA produced by actinobacteria is involved in different pathways enhancing the growth of plant, elongation of roots, cell division and root hair proliferation. A majority of actinobacteria are capable of producing IAA as a secondary metabolite (Pannacci *et al.*, 2022). Different studies suggest that small amounts of IAA is necessary for the growth of primary roots and actinobacteria secreting more than 13.5g/ml of IAA have PGP activity (Duca *et al.*, 2014). Our results also suggests that actinobacterial isolate P2 must be capable of producing IAA for better root development and for overall plant growth promotion. Mesa-Marin *et al.,* (2019) reported that the production of IAA by actinobacteria is dependent on environmental conditions and availability of substrate.

There was a significant suppression in the number of branches of *Solanum lycopersicum* by 55.7% when infected with *X. campestris*, treated plant did not show this suppression and moreover there was a marginal increase in the number of branches. *X. campestris* reduced the fruit productivity to maximum by 44% in *Capsicum frutscens* but the treated plant did not exhibit this reduction. Productivity of fruits was greatly enhanced in *Solanum lycopersicum* whereas *Capsicum frutscens* was not much affected. There was no supression observed in the *Solanum lycopersicum* and *capsicum frutscens* treated with CAE as observed in infected plants whereas there was and increase by 43% in test 1 of *Solanum lycopersicum*.

All the pathogens were found to be restricted by applying culture filtrate of *Streptomycete sp. NCIM 5814*, moreover there was an enhancement of growth and plant productivity observed in the treated plants. The treatment of plants with crude antibiotic extract (CAE) protected the plant from disease caused by the phytopathogen and also improved the plant growth which was found to be better than control 1 which is an untreated plant. The biological activity and the growth promoting effect of our actinobacterial isolate may be due to their ability to produce diverse metabolites like growth harmones that have enhanced the growth of *Solanum lycopersicum* plant. (Moon and Ali, 2022). Additionally, variety of volatile compounds that are produced by *Streptomyces* sp. are also effective against phytopathogens. Lee *et al.,* (2022) reported compounds including dimethyl and trimethyl sulfide from *Streptomyces* sp. AN090126, that inhibited pathogenic bacteria. Dimethyl disulfide produced by actinobacteria alongwith other bioactive compounds is effective against phytopathogen (Polito *et al.*, 2022).

Control 2 was planted to observe the effect of CAE on *Solanum lycopersicum* and *Capsicum frutscens*. All the parameters were found to be positive as compared to the untreated plant which is Control 1. The plant weight of *Capsicum frutscens* increased by 76% and *Solanum lycopersicum* increased by 146% which is a highly encouraging result. Root weight increased by 58 and 21% whereas number of secondary roots increased by 24 and 26% in *Capsicum frutscens* and *Solanum lycopersicum* respectively. Fruit productivity also enhanced greatly in the Control 2 plants *Capsicum frutscens* exhibited enhancement of 76.7% and *Solanum lycopersicum* showed 56.4% increase in productivity. Different actinobacteria have been reported to promote the growth of tomato and chilli plants. Colonization of *Streptomyces rubrolavendulae* S4 strain in tomato and chilli enhanced the survival of plant and also cured it from phytopathogenic infection (Loliam *et al.*, 2012). Srinivas *et al.*, (2020) also observed that *Streptomyces* strains enhanced 18% plant height, 41% shoot weight and 57% fruit yield in tomato plants and in chilli plants plant height, shoot weight and fruit yield was found to be enhanced by 11%, 40% and 50% respectively. Actinobacteria are responsible for the decomposition of organic matter in the environment which contributes to the overall mineral cycling in the nature (Das *et al.*, 2007). Their ability to synthesize lignocellulolytic enzymes can efficiently degrade complex polysaccharides making soil enriched with nutrients. The actinobacteria are capable of adopting in harsh environments and maintain the properties of the soil under balanced condition. These advantages makes

actinobacteria the most preferred microbe for sustainable agricultural practice as they are capable of promoting plant growth and also possess effective biocontrol activity.

Conclusion

The phytopathogens are responsible for the major loss in the agricultural produce. The indiscriminate use of chemicals in the agricultural field for their control effects the soil composition as well as causes toxicity to the living beings consuming it. To sustain the crop yield biological treatment of phytopathogens is an effective method and the present study elucidates that *Streptomyces* sp. NCIM 5814 has exhibited its ability to control the infection caused by *Erwinia carotovora*, *X. campestris* and *X. axonopodis*. This is one shot solution for 3 notorious phytopathogens of very important food crops. The crude extract obtained after submerged fermentation by *Streptomyces* sp. NCIM 5814 protected the *Solanum lycopersicum* and *capsicum frutscens* from phytopathogenic infection as well as improved the plant growth. The ability of actinobacteria to synthesize diverse bioactives, plant growth promoting compounds and enzymes makes them a good alternative to chemical fertilizers and pesticides. *Streptomyces* are abundant source of bioactive compounds with potential application in the agriculture whether it being enhancing the quality of soil, enhancing crop yield or controlling pathogenic infections. Overall these qualities make *Streptomyces* a potential candidate for sustainable agricultural development. Taking these factors into consideration, we can affirm that *Streptomyces* sp. NCIM 5814 is a very promising actinobacteria that can help in promoting ecofriendly crop production and can also enhance the yield of produce. However, further continuous research will allow us to discover more properties of *Streptomyces* sp. NCIM 5814 and its ability to maintain agricultural sustainability to meet the future needs.

Authors Contribution

Saket Mishra has performed the experiments and prepared the manuscript. Tanim Arpit Singh has organised the data and prepared the manuscript. Anjana Jajoo and Sheetal Bhasin designed the experiments and reviewed the data along with the whole manuscript. All the authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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