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Original article

Genetic diversity and antifungal activities of the genera *Streptomyces* and *Nocardiopsis* inhabiting agricultural fields of Tamil Nadu, India

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ABSTRACT

Actinomycetes receives much attention as plant-beneficial bacteria due to their distinct morpho-physiological, plant growth promoting and antifungal properties. Among the actinomycetes, members of the genus *Streptomyces* are of specific interest due to their metabolic versatility and ubiquity. The diversity of *Streptomyces* in different crop fields of Tamil Nadu has not been explored early. In this study, we explored phylogenetic diversity, morphological heterogeneity, and antifungal properties of cultivable actinomycetes isolated from the rhizosphere and bulk soils of different crops collected from 20 distinct districts of Tamil Nadu. A total of 65 actinomycetes were isolated from 40 soil samples including rhizosphere and bulk soils of different crops, and their diversity was analyzed using the 16S rRNA gene sequence. They were then characterized for cultural characteristics and antifungal activity against four fungal strains *Fusarium udum*, *Fusarium oxysporum* f.sp. *ciceri*, *Macrophomina phaseolina* and *Sclerotium rolfsii*. Out of the 65 isolates sequenced, 45 were found to be closely related to *Streptomyces* spp. while the remaining 20 showed similarities with *Nocardiopsis* spp. Cultural characterization on four different ISP media showed immense diversity among the members of the genera *Streptomyces* and *Nocardiopsis*. The strains of *Streptomyces* spp. and *Nocardiopsis* spp. showed varying levels of antifungal activity with all strains found antagonistic against both *Fusarium udum* and *Fusarium oxysporum* f.sp. *ciceris*. All the strains obtained in this study have been accessioned at the National Agriculturally Important Microbial Culture Collection (NAIMCC) to increase the database of characterized strains belonging to these two genera in the collection.

1. Introduction

Actinomycetes are an important group of soil bacteria that are found to be more prevalent in dry soils than wet soils. Soil actinomycetes are known for their plant growth promotional activity as they exhibit various useful traits (Ma et al., 2020). They can also produce a variety of secondary metabolites, many of which are antibacterial or antifungal (Olanrewaju and Babalola, 2019). Most antibiotics used in human medicine are metabolites of actinomycetes, with many of them originating from *Streptomyces* spp. (Ma et al., 2020). The ability to synthesize a variety of chemical substances such as antibiotics, enzymes, and anti-tumor medicines, made *Streptomyces* spp. most common genus used in the pharma industry (Chaudhary et al., 2013). In soil, they grow as substrate mycelium and produce a range of enzymes including chitinase, xylanase and cellulase that breakdown complex organic polymers into constituent simple sugars (Seipke et al., 2012) thereby playing a direct role in biogeochemical cycles. They are significant candidates for natural fertilizers because of their ability to cycle nutrients. They are effective colonizers of rhizosphere and rhizoplane tissues with the potential to enhance plant growth, and development and boost yield (Dias et al., 2017). *Streptomyces* spp. promote plant growth through the production of phytohormones like auxins, cytokinins, and gibberellins, additionally they produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase whose activity is important in the suppression of plant stress

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(Sadeghi et al., 2012; Verma et al., 2011). They are implicated in phosphate solubilization (Jog et al., 2014) and siderophore production (Verma et al., 2011) as well. Yet the most important role of *Streptomyces* spp. in agriculture is their ability to control phytopathogens owing to their traits; siderophore production, antibiotics production and volatile compounds secretion (Olanrewaju and Babalola, 2019).

Isolation, morphological, biochemical, physiological, and molecular characterization of *Streptomyces* spp. are reported in many studies (Taddei et al., 2006). Taddei et al. (2006) studied the biochemical and morphological characterization of 71 *Streptomyces* spp. isolated from soil samples of Venezuela, of them 67 isolates were new strains. In a recent study, Kaur et al. (2019) tested a potent *Streptomyces* strain MR14 with antifungal and plant growth promoting properties. Cell free extracts of MR14 showed potent antagonistic activity against 13 different fungal phytopathogens. Despite their multifarious role, *Streptomyces* found beneficial to plants and agriculture overall, received lesser interest as compared to *Streptomyces* spp. studied for their role in pharmaceuticals (Rey and Dumas, 2017). It is only imperative, that the exploration of the phylogenetic and morphological diversity of this group of bacteria and enriching their cultural database could lead to identifying strains with unique potential. Agricultural fields especially plant rhizosphere is an important source of potent *Streptomyces* spp. with antagonistic activity against plant pathogens. In the present study, an attempt was made to isolate *Streptomyces* spp. from different crop rhizosphere and bulk soil samples collected from Tamil Nadu, India. The resultant isolates were characterized for morphological diversity, genetic diversity, and antagonistic potential against select phytopathogenic fungi. All the actinomycetes isolated in this study were submitted to the National Agriculturally Important Microbial Culture Collection (NAIMCC), a constituent unit of ICAR-National Bureau of Agriculturally Important Microorganisms, Mau India.

2. Materials and methods

2.1. Sample collection

A total of 40 soil samples including rhizosphere of different crops and bulk soils were collected in sterile bags from various locations in Tamil Nadu, India, details of the samples are depicted in Table 1. For rhizosphere samples, standing crops were uprooted at five random sites in a field and soils adhered to the roots were collected in a sterile poly bag. For bulk soil, the top 15 cm soil core was collected from five random sites in a sterile poly bag from each site. The samples once brought to the laboratory were shade dried aseptically and stored in a cold room (4 °C) until further processing.

2.2. Isolation of actinomycetes from soil

A selective media containing basal nutrients and antibiotics was used for the isolation of *Streptomyces*. Starch casein agar (SCA) containing soluble starch 10 gL^{-1}, casein 0.3 gL^{-1}, KNO_3 2.0 gL^{-1}, $MgSO_4{\cdot}7H_2O$ 0.05 gL^{-1}, K_2HPO_4 2.0 gL^{-1}, NaCl 2.0 gL^{-1}, $CaCO_3$ 0.02 gL^{-1}, $FeSO_4{\cdot}7H_2O$ 0.01 gL^{-1} and Agar 20.0 gL^{-1} supplemented with cycloheximide (50 mg/L), nystatin (50 mg/L) and rifampicin (50 mg/L) was used as the selective media (Nolan and Cross, 1988). The pH of the media was adjusted to 7. Standard serial dilution and plating method was followed to isolate *Streptomyces*. Aliquots (100 µL) of dilutions, 1/1000 and 1/10000 were plated on the selective media and incubated at 30 ± 1 °C for 7–10 days. Plates were observed regularly for the growth of the actinomycetes population. Different morphotypes from each of the samples were picked and purified in the selective media. The isolates were then stored as glycerol stocks (20 %) at −80 °C and slants at 4 °C until further study.

2.3. DNA isolation and 16S rRNA gene sequence amplification

Total DNA was extracted from fully grown actinomycetes cultures using Nucleo-pore gDNA Fungal Bacterial Mini Kit employing the manufacturer's protocol. The quality of DNA was checked and ensured both by gel electrophoresis and nanodrop. Universal primers pA (AGAGTTTGATCCTGGCTCAG) and pH (AAGGAGGTGATCCAGCCGCA) were used for the amplification of the 16S rRNA gene. The PCR reactions were carried out in a peqSTAR thermal cycler by following the protocols of Kumar et al. (2014) with slight modifications. Briefly, the reactions were carried out in 25 µL volume comprising 12.5 µL Go Taq green Master Mix 2X (Promega, USA), 100 ng of primers (pA and pH), 50 ng DNA template and nuclease free water to adjust the volume. PCR conditions used were as follows; initial denaturation-95 °C for 5 min followed by 35 cycles of denaturation-95 °C for 30 s, annealing-52 °C for 40 s and extension-72 °C for 60 s; and a final extension at 72 °C for 10 min. The amplified PCR products were visualized in 1.5 % agarose gel electrophoresis (stained with ethidium bromide) using a gel documentation system (Bio Rad, USA).

2.4. 16S rRNA gene sequencing and phylogenetic analysis

PCR products were purified using Gene JET PCR Purification Kit (Thermo Fisher, USA) and sequenced using the primer 1100R (GGGTTGCGCTCGTTG) (Engelbrektson et al., 2010) at GeneMatrix sequencing facility by following sanger dideoxy sequencing method in an ABI 3130xl Genetic Analyzer. Resultant sequences were then quality filtered in FinchTV and trimmed for better quality sequences (Stucky, 2012). Quality ensured sequences were then used to run a BLAST search against 16S rRNA gene sequences of type species available in EZ Biocloud to identify the isolates (https://www.ezbiocloud.net). Sequences were aligned and compared using the multiple sequence alignment tool, CLUSTAL W and a phylogenetic tree was constructed employing the neighbour joining method (Saitou and Nei, 1987). The Program used for phylogenetic tree construction is MEGA X (Kumar et al., 2018).

2.5. Characterization of soil actinomycetes based on pigment formation, growth pattern and mycelium formation

Cultural characteristics of all the actinomycete isolates were examined on four different ISP (International Streptomyces Project) media *viz*., ISP1, 2, 3 and 7, incubated at 28 ± 2 °C for 14 days. Morphological characteristics like pigment formation, growth pattern (recorded under three categories *viz*., poor, moderate and Good) and the colour of aerial and substrate mycelium were recorded.

2.6. Antifungal activity against phytopathogenic fungi

Four phytopathogenic fungi *viz*., *Fusarium udum* (NAIMCC-F-02017), *Fusarium oxysporum* f. sp. *ciceris* (NAIMCC-F-00889), *Macrophomina phaseolina* (NAIMCC-F-01260) and *Sclerotium rolfsii* (NAIMCC-F-01638) were collected from NAIMCC. They were grown on potato dextrose agar plates with an incubation at 28 ± 2 °C for 24–120 h and maintained on PDA slants and mineral oil for further use. Freshly grown cultures were used for antifungal assays. A dual culture assay was employed to test the antagonistic activity of *Streptomyces* spp. against four selected phytopathogens. SCA and PDA in the ratio of 1:1 was used as a medium to test the antagonistic activity. Fresh cultures of the bacterium were inoculated on two

sides of the plate with the selected medium. The centre of the plate was inoculated with fresh mycelium of the test pathogens and the plates were incubated at 28 ± 2 °C for 96 h. Plates were observed twice a day during incubation for inhibition zones. Plates inoculated with test pathogen alone were also incubated simultaneously as a control. The zone of inhibition and percent inhibition of fungi were calculated using the following formula.

$$\text{Zone of inhibition} = F - A$$

$$\text{Percent inhibition (PI)} = [(F - A)/F] \times 100$$

where F = radius of fungal growth from the centre (cm) in the control plate.

A = the radius of radial fungal growth from the centre (cm) in the dual culture plate.

3. Results

3.1. Actinomycetes community composition

Isolation on the selective media resulted in a total of sixty-five isolates from 40 samples collected from Tamil Nadu (Table 1). Identification based on 16S rRNA gene sequencing and blast search against type species database revealed that these 65 isolates were affiliated to two genera belonging to *Streptomyces* and *Nocardiopsis* (Fig. 1).

3.2. Streptomyces

The genus *Streptomyces* represents 45 isolates accounting for 67.69 % of the total isolates. It was represented by 18 species *viz*., *S griseus* (4), *S. anulatus* (3), *S. microflavus* (2*), S. covourensis* (1), *S. araujoniae* (3), *S. zhihengii* (1), *S. roseofulvus* (3), *S. daghestanicus* (5), *S. carpaticus* (1), *S. matensis* (2), *S. glaucescens* (1), *S. tendae* (3), *S. nigra* (1), *S. albogriseolus* (1), *S. pilosus* (1), *S. rochei* (3), *S. bohaiensis* (8), *S. lonarensis* (2).

3.3. Nocardiopsis

Identified isolates include *Nocardiopsis* representing 32.31 % of the total isolates. Isolates belonging to *Nocardiopsis* are not as diverse as *Streptomyces* representing only three species *viz*., *Nocardiopsis dassonvillei* (11), *Nocardiopsis flavescens* (8) and *Nocardiopsis listeria* (1).

3.4. Phylogenetic diversity of actinomycetes based on 16S rRNA gene sequences

The relationship between the two genera obtained in the present study demonstrated through a phylogenetic tree is presented in Fig. 1. All the 65 strains were grouped into two genera *Streptomyces* and *Nocardiopsis*; the upper side of the dendrogram representing *Streptomyces* with 18 species, *S. griseus*, *S. anulatus*, *S. microflavus, S. covourensis*, *S. araujoniae*, *S. zhihengii*, *S. roseofulvus*,

Table 1
Details of samples and actinomycetes isolated in the study.

GPS location	Address	Sample type	Strain no
9°53′22.0″N 78°09′22.0″E	Chinthamani, District Madurai, Tamil Nadu	Bulk soil coconut field	TN1, TN2
9°40′9.0″N 78°05′34.0″E	Kariapatti, District Virudhunagar, Tamil Nadu	Bulk soil after harvest of maize	TN3, TN4
9°29′37.0″N 78°06′25.0″E	Aruppukkottai, District Virudhunagar, Tamil Nadu	Bulk soil castor field	TN5, TN7
9°23′30.0N 78°07′40.0 E	Pandalgudi, District Virudhunagar, Tamil Nadu	Sunflower rhizosphere	TN6, TN8
9°08′50.0″N 78°00′23.0″E	Ettaiyapuram, District Thoothukkudi, Tamil Nadu	Bulk soil Jatropha field	TN9, TN10
8°51′04.0″N 78°07′15.0″E	Thoothukudi, Thoothukkudi District, Tamil Nadu	Sorghum rhizosphere	TN11, TN12
9°00′24.0″N 78°12′04.0″E	Vaippar, District Thoothukkudi, Tamil Nadu	Chilli rhizosphere	TN13, TN14
9°07′13.0″N 78°24′32.0″E	Sayalkudi, District Ramanathapuram, Tamil Nadu	Palm field bulk soil	TN15, TN16
9°13′17.0″N 78°36′37.0″E	Keerandai, District Ramanathapuram,Tamil Nadu	Rice rhizosphere	TN17, TN18
9°18′01.0″N 78°49′19.0″E	Pallamerkkulam, District Ramanathapuram, Tamil Nadu	Rice rhizosphere	TN19, TN20
9°22′57.0″N 78°50′08.0″E	Veethi, District Ramanathapuram, Tamil Nadu	Rice rhizosphere	TN21, TN22
9°37′21.0″N 78°55′50.0″E	Uppur, District Ramanathapuram, Tamil Nadu	Rice rhizosphere	TN23, TN24
10°03′58.0″N 79°13′48.0″E	Mumpalai, District Pudukkottai Tamil Nadu	Rice rhizosphere	TN25, TN26
10°18′10.0″N 79°20′27.0″E	Chinnaavudaiyarkoil, District Thanjavur, Tamil Nadu	Rice rhizosphere	TN27, TN28
10°25′32.0″N 79°32′52.0″E	Udayamarthandapuram, District Thiruvarur, Tamil Nadu	Rice rhizosphere	TN29, TN30
10°25′09.0″N 79°47′33.0″E	Kuravapulam, Nagapattinam District, Tamil Nadu	Rice rhizosphere	TN31, TN32
10°37′47.0″N 79°47′08.0″E	Meenamanallur, Nagapattinam District, Tamil Nadu	Rice rhizosphere	TN33, TN34
11°06′06.0″N 79°49′57.0″E	Chinnamedu, Mayiladuthurai District, Tamil Nadu	Rice rhizosphere	TN35, TN36
11°13′36.0″N 79°43′24.0″E	Sirkali, Mayiladuthurai District, Tamil Nadu	Rice rhizosphere	TN37, TN38
11°30′45.0″N 79°43′11.0″E	Chinnakomatti, District Cuddalore, Tamil Nadu	Rice rhizosphere	TN39, TN40
12°43′37.0″N 80°11′23.0″E	Thiruporu, District Chengalpattu, Tamil Nadu	Rice rhizosphere	TN41, TN42
12°53′48.0″N 80°14′50.0″E	Panaiyur, District Chennai, Tamil Nadu	Bulk soil coconut field	TN43, TN44
12°39′32.0″N 79°57′01.0″E	Grand Southern Trunk Road, Chengalpattu District Tamil Nadu	Rice rhizosphere	TN45, TN46
12°22′20.0″N 79°47′39.0″E	Kadamalaiputhur, District Kanchipuram, Tamil Nadu	Groundnut rhizosphere	TN47, TN48
12°11′02.0″N 79°37′25.0″E	Tindivanam, District Viluppuram, Tamil Nadu	Bhendi rhizosphere	TN49, TN50
11°40′51.0″N 79°17′18.0″E	Ulundurpet, District Kallakurichi, Tamil Nadu	Grassland	TN51
11°30′47.0″N 79°06′03.0″E	Kalpadi North, District Perambalur, Tamil Nadu	Brinjal rhizosphere	TN52
11°12′42.0″N 78°52′56.0″E	Pichandarkovil, District Tiruchirappalli, Tamil Nadu	Rice rhizosphere	TN53
10°54′11.0″N 78°43′30.0″E	Melapachchakudi, District Pudukkottai, Tamil Nadu	Rice rhizosphere	TN54
10°39′33.0″N 78°35′33.0″E	Kallupatti, District Tiruchirappalli, Tamil Nadu	Sorghum rhizosphere	TN55
10°25′40.0″N 78°26′07.0″E	Karungalakudi, District Madurai, Tamil Nadu	Brinjal rhizosphere	TN56
10°08′35.0″N 78°21′17.0″E	Kalikulam, District Tirunelveli, Tamil Nadu	Rice rhizosphere	TN57
9°59′50.0″N 78°15′23.0″E	Kalligudi, District Madurai, Tamil Nadu	Rice rhizosphere	TN58
9°43′33.0″N 77°58′46.0″E	Maniparaipatti, District Virudhunagar, Tamil Nadu	Redgram rhizosphere	TN59
9°25′23.0″N 77°55′08.0″E	Tenkasi, District Tenkasi, Tamil Nadu	Maize rhizosphere	TN60
9°12′41.0″N 77°47′45.0″E	Sankarankovil, District Tenkasi, Tamil Nadu	Rice rhizosphere	TN61
9°15′24.0″N 77°39′57.0″E	Alagiapandiapuram,District Tirunelveli, Tamil Nadu	Lemon rhizosphere	TN62
8°55′33.0″N 77°38′41.0″E	Palayamkottai, District Tirunelveli, Tamil Nadu	Rice rhizosphere	TN63
8°44′38.0″N 77°44′54.0″E	Amarapuram, District Thoothukudi, Tamil Nadu	Bulk soil banana field	TN64
8°32′31.0″N 78°06′43.0″E	Amarapuram, District Thoothukudi, Tamil Nadu	Bulk soil coconut field	TN66

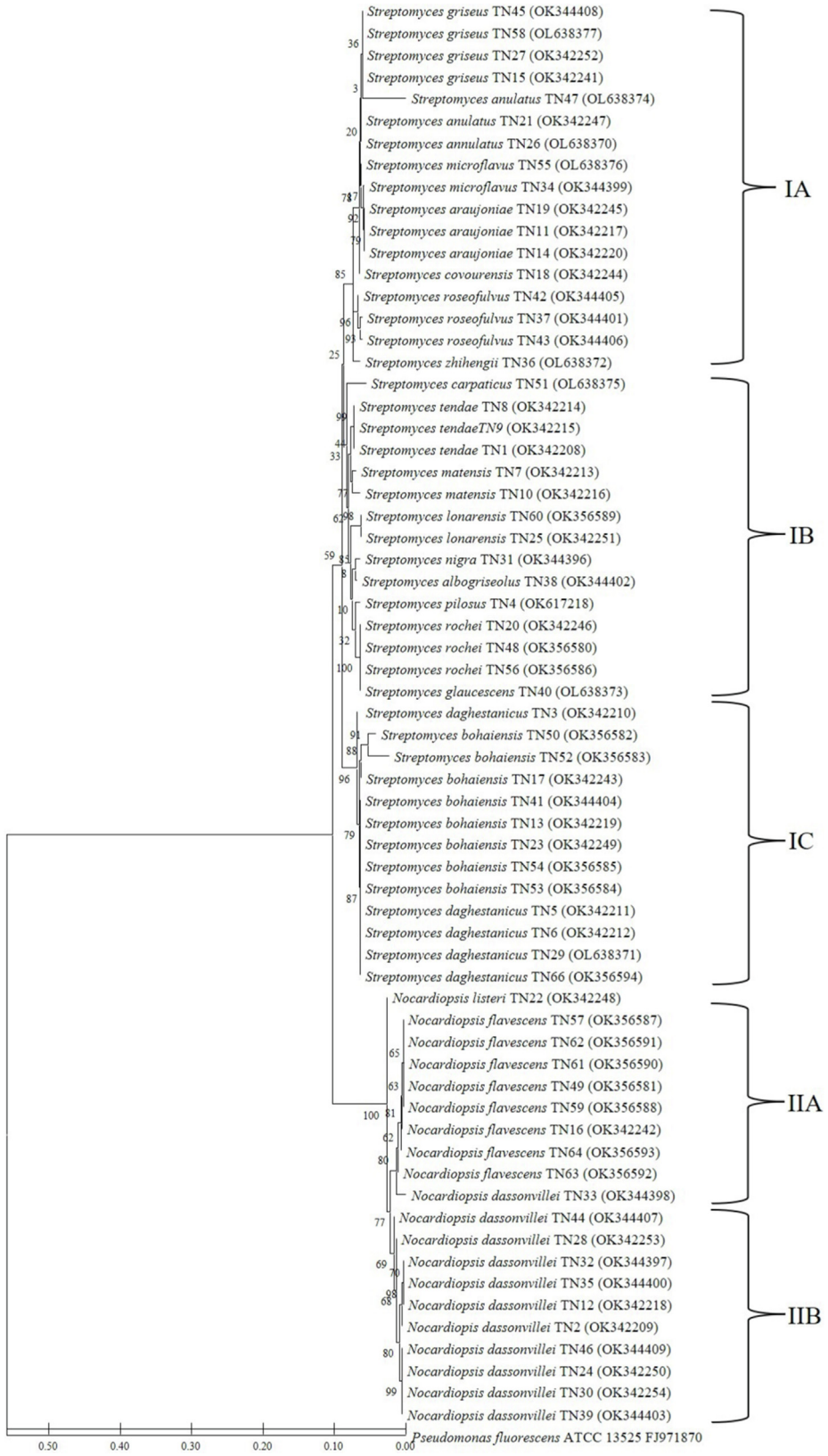

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the 45 *Streptomyces* and 20 *Nocardiopsis* strains. Bootstrap value based on 1000 resampled datasets are shown at branch nodes.

S. daghestanicus, *S. carpaticus*, *S. matensis*, *S. glaucescens*, *S. tendae*, *S. nigra*, *S. albogriseolus*, *S. pilosus*, *S. rochei*, *S. bohaiensis* and *S. lonarensis*. *Streptomyces griseus*, *S. anulatus*, *S. microflavus* and *S. araujoniae* separate in sub cluster 1A; *Streptomyces griseus* showed more close similarity with *S. anulatus*. *Streptomyces covourensis*, *S. zhihengii*, *S. carpaticus*, *S. matensis*, *S. glaucescens*,

S. nigra, *S. albogriseolus*, and *S. pilosus*, were represented by single nodes which separate in different sub cluster. The larger cluster was represented by *Streptomyces bohaiensis* which separates in subcluster 1C, which is also represented by *S. daghestanicus*. Major cluster 2 is represented by the genus *Nocardiopsis* and it has been divided into two sub clusters, 2A and 2B. Sub cluster 2A presents all *Nocardiopsis flavescens* and single node *Nocardiopsis listeria* while sub cluster 2B represents all *Nocardiopsis dassonvillei*.

The phylogenetic tree presenting the relationship among the 45 strains of the genus *Streptomyces* is presented in Fig. 2. It showed the grouping of sequences into three distinct groups I, II, and III. Group I present a maximum of 17 *Streptomyces* strains; all three strains of *S.araujoniae* isolated from three different crop fields which showed 99 % sequence similarity formed a distinct branch in this group. Similarly, in this group *S. griseus*, *S. anulatus*, *S. microflavus* and *S. roseofulvus* forms separate cluster; these were isolated from different crop fields and showed more than 99 % sequence similarities. One strain of *S. covourensis* and *S. zhihengii* in cluster I were collected from the rice rhizosphere. Two strains of *S. lonarensis* separated in cluster II which showed 99–100 % similarity with respective species and were collected from rice and maize rhizosphere. Interestingly major cluster II comprises 15 strains separated into three subclusters, with a maximum of eight strains that belong to *S. bohaiensis*, followed by *S. daghestanicus*. Cluster III presents five species with single isolates of each which includes *S. glaucescens*, *S. pilosus*, *S. nigra*, *S. albogriseolus* and *S. carpaticus*; these strains were collected from rice, bulk soil, and grass field as presented in Table 1. Additionally, this cluster also presents 3 strains each of *S. rochei* and *S. tendae*; all these six strains were isolated from six different crop fields. Fig. 3 presents the relationship among the members of *Nocardiopsis* which exhibits the grouping of sequences in two major groups; cluster I comprise a maximum of 10 strains which showed 99–100 % sequence similarities with *Nocardiopsis dassonvillei*. Out of these 10 strains, seven were isolated from rice rhizosphere, two from coconut fields and one from sorghum rhizosphere. Further cluster II comprises eight strains that showed 99 % sequence similarities with *Nocardiopsis flavescens* these isolates were from six different crop fields which includes palm, okra, rice, redgram, lemon, and banana. Additionally in this cluster, 1 strain showed 99 % sequence similarities with *Nocardiopsis listeri* isolated from rice rhizosphere grouped as a separate subgroup.

3.5. Morphological characteristics of actinomycetes

The morphological properties of all 65 actinomycetes strains are presented in Supplementary Table 1. The strains showed differential growth patterns on different ISP media. Six strains viz., *S. daghestanicus* TN6, *S. araujoniae* TN14, *N. flavescens* TN14, *S.bohaiensis* TN17, *S. griseus* TN27 and *N. dassonvillei* TN33 were characterized as showing good growth in all the four ISP media. Twenty-one strains were characterized as showing good growth in atleast three ISP media while seventeen strains showed good growth in atleast two ISP media. Pigment production was observed in twenty strains in atleast one media. *S. zhihengii* TN 36 produced pigment on all four ISP media and *S. araujoniae* TN19 produced pigments on three ISP media while the strains *S. araujoniae* TN11, *S. araunoniae* TN14 and *S. anulatus* TN55 produced pigments on atleast two ISP media. The Media ISP-3 facilitated the production of pigment by 18 strains TN1, TN3, TN6, TN8, TN9, T11, TN13, TN14, TN16, TN17, TN19, TN28, TN31, TN35, TN38, TN55, TN63, and TN66; Six strains which include TN1, TN8, TN9, TN11, TN19, and TN28, exhibit pigment formation on ISP-1 media. Five strains showed pigmentation on ISP-4 media which includes TN4, TN5, TN9, TN14, and TN19 while ISP-7 facilitated pigment formation by only one strain (TN9). The colour of aerial and substrate mycelium of all the 65 actinomycetes strains was also recorded which includes white, brown, pale green, pale yellow, yellow, grey, greenish-yellow, light green, green, light yellow and dark green.

3.6. Antifungal activity of actinomycetes strains

Antifungal activities of all the 65 actinomycetes strains against four fungal strains, *F. udum*, *F. oxysporum* f.sp. *ciceris, M. phaseolina* and *S. rolfsii.* are represented in Table 3. All 65 strains showed antifungal activity against *F. udum* with inhibition ranging from 1.96 to 63.99 %. *S. daghestanicus* TN3, *N. dassonvillei* TN12 and *S. araujoniae* TN19 showed maximum inhibition with respective inhibition percentages of 63.99, 63.96 and 63.96. Twenty strains showed more than 50 % inhibition against *F. udum*. Similarly, all the strains exhibited antifungal activity against *F. oxysporum* f.sp. *ciceris* with inhibition ranging from 1.79 to 63.81 %. Nine strains showed more than 50 % inhibition against *F. oxysporum* while strains *S. araujoniae* TN11 and *S. daghestanicus* TN3 showed maximum inhibition with a percent inhibition of 63.81 and 61.33, respectively. Out of 65 strains, 48 showed the ability to inhibit *Macrophomina phaseolina* on plates with inhibitions ranging from 3.75 to 82.50 %. Twenty strains in the study have shown inhibition percent of 70 and above against *M. phaseolina* while strains *S. rochei* TN56, *N. flavescens* TN49 and *S. daghestanicus* TN 66 showed maximum inhibitions with percent inhibitions of 82.50, 81.67 and 79.17 respectively. Against *S. rolfsii* 39 strains showed inhibition and inhibition percent was recorded between 3.33 and 52.50. Only three strains *viz*., *S. araujoniae* TN11, *S. araujoniae* TN19 and *S. griseus* TN27 showed more than 40 % inhibition. A total of 27 strains showed antifungal activity against all four fungal strains, with six strains *viz*., *S. pilosus* TN4 (Fig. 4), *S. tendae* TN8, *S. araujoniae* TN11, *N. dassonvillei* TN12 *S. araujoniae* TN19 and *S. griseus* TN27 showing considerable percent inhibition against all the four pathogens tested, while 33 strain showed antifungal activity against atleast three fungal strains.

3.7. Nucleotide sequence accession numbers

The 16S rRNA gene sequences of 65 strains identified in the current study have been submitted to the GenBank nucleotide sequence database (https://www.ncbi.nlm.nih.gov/nucleotide). All the strains isolated and characterized in this study were submitted to NAIMCC (National Agriculturally Important Microbial Culture Collection, ICAR-NBAIM, India (Table 2).

4. Discussion

Actinomycetes are endowed with metabolic potential to survive and prosper in varied environments including agricultural fields where their presence impacts the survival and growth of other bacteria, as well as plants (Verma et al., 2011; Sadeghi et al., 2012; Bennur et al., 2016; Olanrewaju and Babalola, 2019; Ma et al., 2020). Among the actinomycetes, the genus *Streptomyces* is endowed with a plethora of metabolic capabilities and hence considered as bacteria of immense importance both in natural environments and Industry. To explore the potential of this group of bacteria it is necessary to understand their diversity, physiology, and ecology (Ma et al., 2020). The aim of this study was to specifically isolate *Streptomyces* and study their genetic and cultural diversity and explore their antifungal potential against various phytopathogens. A selective media was used to isolate *Streptomyces* spp. from crops' rhizosphere and bulk soil collected from different fields and different regions of Tamil Nadu, India. A total of 65 putative *Streptomyces* isolates were collected, characterized, and identified. Identification based on 16S rRNA gene sequence similarity revealed the isolates fall under two genera *viz*., *Streptomyces*

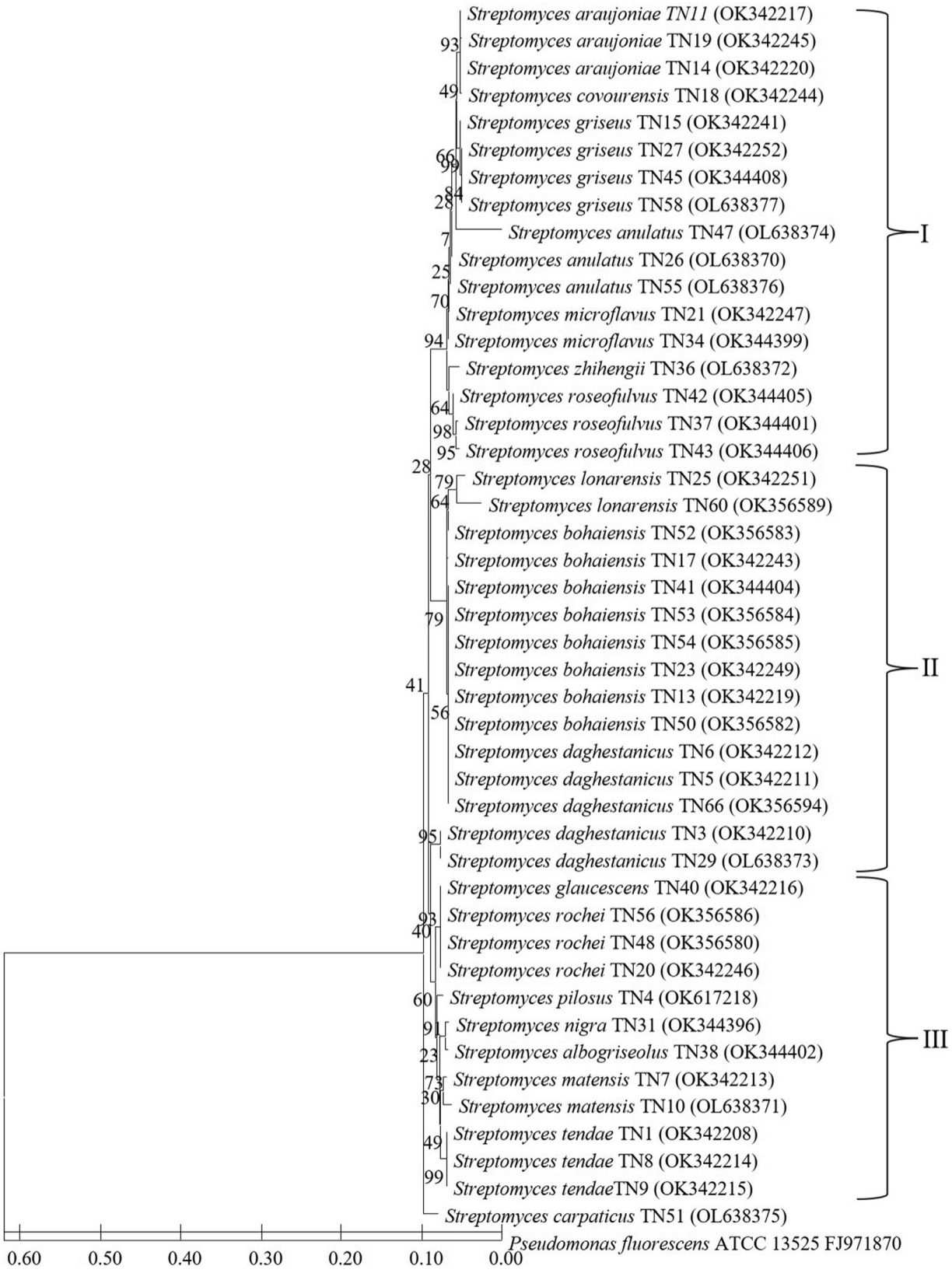

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship among the 45 *Streptomyces* strains. Bootstrap value based on 1000 resampled datasets are shown at branch nodes.

and *Nocardiopsis*. It's worth noting that the 16S rRNA gene sequence's utility as a phylogenetic and taxonomy identifier is restricted. The core genome divergence between *Streptomyces* strains with 97 percent 16S rRNA gene sequence identity can be as high as 30 %, with an ANI of 100–78.3 % (van Bergeijk et al., 2020). 16S rRNA gene sequence identity of 98.6 % is widely used

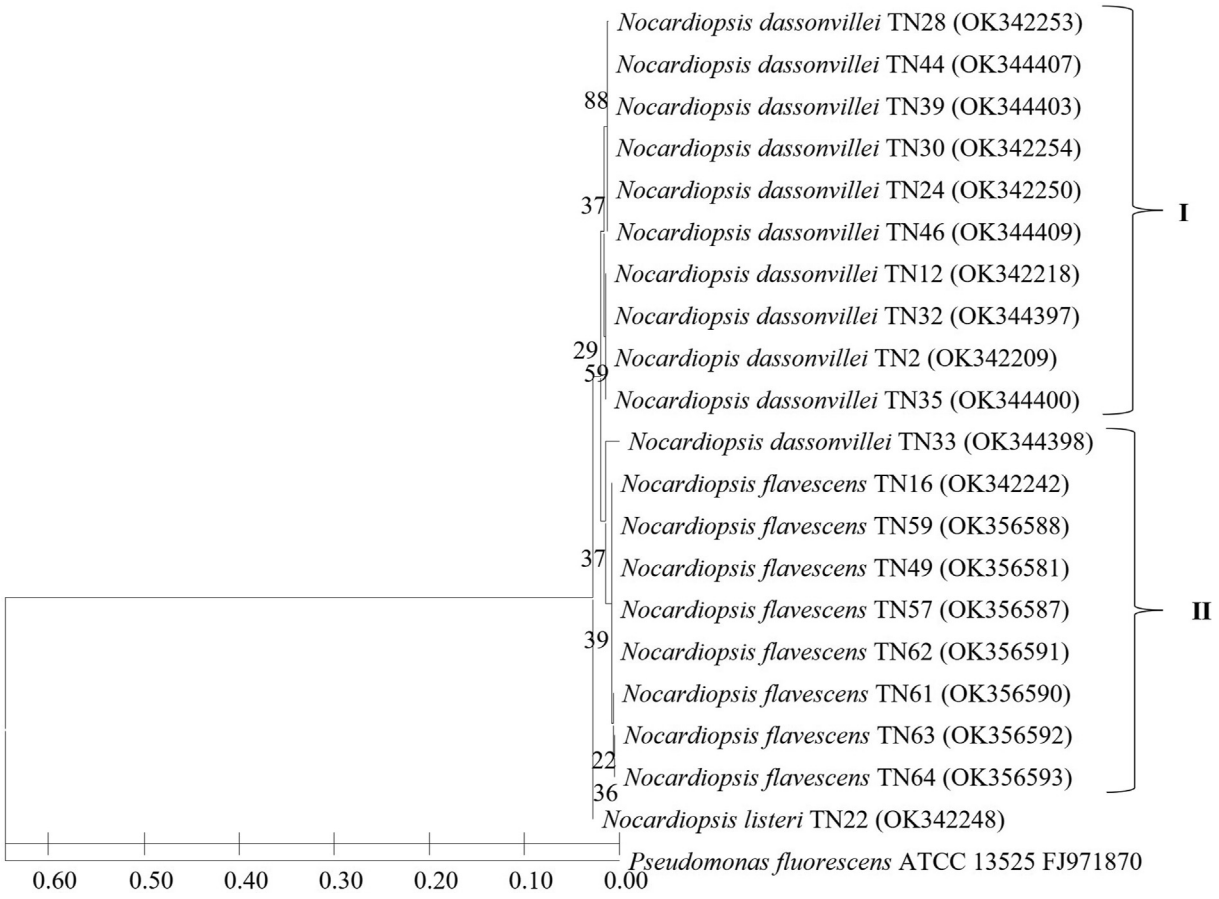

Fig. 3. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship among the 20 *Nocardiopsis* strains. Bootstrap value based on 1000 resampled datasets are shown at branch nodes.

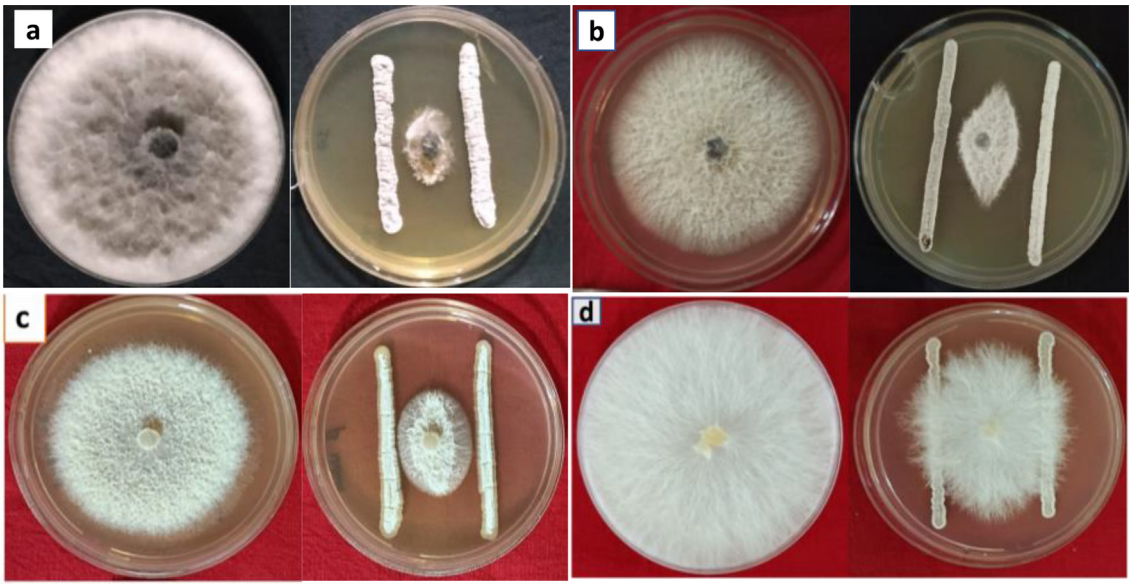

Fig. 4. Antifungal activity of *Streptomyces pilosus* TN4 against different fungal pathogens; *Macrophomina phaseolina* (a), *Fusarium oxysporum* (b), *Fusarium udum* (c) and *Sclerotium rolfsii* (d).

for bacterial species identification and delineation (Chun et al., 2018); however, for actinomycetes, this threshold is raised to 99 % based on comparative studies between 16S rRNA gene sequences (Guo et al., 2015). As a result, we used a 99–100 % threshold to assign actinomycetes isolates to distinct clusters. Since more than 30 % of the isolates obtained in the study belonged to *Nocordiopsis* we have taken those isolates too in further analysis. Like *Streptomyces* spp. members of the genus *Nocardiopsis* too can

Table 2
Identities of actinomycetes isolated in this study, NCBI accession numbers of 16S rRNA gene sequence and NAIMCC accessions numbers.

Strain No	Identity	NAIMCC accession number	NCBI accession number
TN1	*Streptomyces tendae*	NAIMCC-B-02880	OK342208
TN2	*Nocardiopis dassonvillei*	NAIMCC-B-02865	OK342209
TN3	*Streptomyces daghestanicus*	NAIMCC-B-02874	OK342210
TN4	*Streptomyces pilosus*	NAIMCC-B-03007	OK617218
TN5	*Streptomyces daghestanicus*	NAIMCC-B-02875	OK342211
TN6	*Streptomyces daghestanicus*	NAIMCC-B-02876	OK342212
TN7	*Streptomyces matensis*	NAIMCC-B-02878	OK342213
TN8	*Streptomyces tendae*	NAIMCC-B-02881	OK342214
TN9	*Streptomyces tendae*	NAIMCC-B-02882	OK342215
TN10	*Streptomyces matensis*	NAIMCC-B-02879	OK342216
TN11	*Streptomyces araujoniae*	NAIMCC-B-02868	OK342217
TN12	*Nocardiopsis dassonvillei*	NAIMCC-B-02866	OK342218
TN13	*"Streptomyces bohaiensis*	NAIMCC-B-02871	OK342219
TN14	*Streptomyces araujoniae*	NAIMCC-B-02869	OK342220
TN15	*Streptomyces griseus*	NAIMCC-B-02877	OK342241
TN16	*Nocardiopsis flavescens*	NAIMCC-B-02867	OK342242
TN17	*Streptomyces bohaiensis*	NAIMCC-B-02872	OK342243
TN18	*Streptomyces covourensis*	NAIMCC-B-02873	OK342244
TN19	*Streptomyces araujoniae*	NAIMCC-B-02870	OK342245
TN20	*Streptomyces rochei*	NAIMCC-B-02904	OK342246
TN21	*Streptomyces microflavus*	NAIMCC-B-02901	OK342247
TN22	*Nocardiopsis listeri*	NAIMCC-B-02908	OK342248
TN23	*Streptomyces bohaiensis*	NAIMCC-B-02897	OK342249
TN24	*Nocardiopsis dassonvillei*	NAIMCC-B-02889	OK342250
TN25	*Streptomyces lonarensis*	NAIMCC-B-02900	OK342251
TN26	*Streptomyces anulatus*	NAIMCC-B-02894	OL638370
TN27	*Streptomyces griseus*	NAIMCC-B-02899	OK342252
TN28	*Nocardiopsis dassonvillei*	NAIMCC-B-02890	OK342253
TN29	*Streptomyces daghestanicus*	NAIMCC-B-03002	OL638371
TN30	*Nocardiopsis dassonvillei*	NAIMCC-B-02891	OK342254
TN31	*Steptomyces nigra*	NAIMCC-B-02903	OK344396
TN32	*Nocardiopsis dassonvillei*	NAIMCC-B-02892	OK344397
TN33	*Nocardiopsis dassonvillei*	NAIMCC-B-02893	OK344398
TN34	*Streptomyces microflavus*	NAIMCC-B-02902	OK344399
TN35	*Nocardiopsis dassonvillei*	NAIMCC-B-02894	OK344400
TN36	*Streptomyces zhihengii*	NAIMCC-B-03010	OL638372
TN37	*Streptomyces roseofulvus*	NAIMCC-B-02905	OK344401
TN38	*Streptomyces albogriseolus*	NAIMCC-B-02896	OK344402
TN39	*Nocardiopsis dassonvillei*	NAIMCC-B-02895	OK344403
TN40	*Streptomyces glaucescens*	NAIMCC-B-03003	OL638373
TN41	*Streptomyces bohaiensis*	NAIMCC-B-02898	OK344404
TN42	*Streptomyces roseofulvus*	NAIMCC-B-02906	OK344405
TN43	*Streptomyces roseofulvus*	NAIMCC-B-02907	OK344406
TN44	*Nocardiopsis dassonvillei*	NAIMCC-B-02985	OK344407
TN45	*Streptomyces griseus*	NAIMCC-B-03004	OK344408
TN46	*Nocardiopsis dassonvillei*	NAIMCC-B-02986	OK344409
TN47	*Streptomyces anulatus*	NAIMCC-B-02995	OL638374
TN48	*Streptomyces rochei*	NAIMCC-B-03008	OK356580
TN49	*Nocardiopsis flavescens*	NAIMCC-B-02992	OK356581
TN50	*Streptomyces bohaiensis*	NAIMCC-B-02997	OK356582
TN51	*Streptomyces carpaticus*	NAIMCC-B-03001	OL638375
TN52	*Streptomyces bohaiensis*	NAIMCC-B-02998	OK356583
TN53	*Streptomyces bohaiensis*	NAIMCC-B-02999	OK356584
TN54	*Streptomyces bohaiensis*	NAIMCC-B-03000	OK356585
TN55	*Streptomyces anulatus*	NAIMCC-B-02996	OL638376
TN56	*Streptomyces rochei*	NAIMCC-B-03009	OK356586
TN57	*Nocardiopsis flavescens*	NAIMCC-B-02987	OK356587
TN58	*Streptomyces griseus*	NAIMCC-B-03005	OL638377
TN59	*Nocardiopsis flavescens*	NAIMCC-B-02988	OK356588
TN60	*Streptomyces lonarensis*	NAIMCC-B-03006	OK356589
TN61	*Nocardiopsis flavescens*	NAIMCC-B-02989	OK356590
TN62	*Nocardiopsis flavescens*	NAIMCC-B-02990	OK356591
TN63	*Nocardiopsis flavescens*	NAIMCC-B-02991	OK356592
TN64	*Nocardiopsis flavescens*	NAIMCC-B-02993	OK356593
TN66	*Streptomyces daghestanicus*	*Submitted to NAIMCC	OK356594

survive under different environmental conditions and produce a range of bioactive compounds (Bennur et al., 2016). In the current study, 45 isolates were assigned to *Streptomyces* spp., and 20 isolates to *Nocardiopsis* spp. based on 16S rRNA gene sequences. *Streptomyces* spp. is reported to have advanced adaptability to exist in different extreme environments and habitant of diverse conditions like frozen soils, deserts, and oceans (Okoro et al., 2009; Passari et al., 2018). The findings of this study have shown that there is a high level of diversity within *Streptomyces* spp. present in the agriculture fields of Tamil Nadu. The isolates collected from different crop rhizosphere and bulk soils of agriculture fields fell under 18 species forming three clades. A similar study on the diversity of streptomycetes in prairie soils resulted in 34 different OTUs, albeit the 16S rRNA gene sequence used for diversity analy-

Table 3
Antifungal activity of 65 actinomycetes strains against four fungal strains.

Strain	*F. udum*	*F. oxysporum*	*Macrophomina phaseolina*	*S. rolfsii*
TN1	51.04 ± 9.11	12.50 ± 0.634	40.00 ± 2.04	14.17 ± 0.393
TN2	52.02 ± 1.89	42.38 ± 2.36	71.67 ± 3.12	20.83 ± 1.42
TN3	63.99 ± 2.53	61.33 ± 2.20	63.33 ± 3.12	0.00
TN4	49.05 ± 4.28	51.79 ± 3.78	75.00 ± 4.08	38.33 ± 2.47
TN5	29.03 ± 3.06	5.36 ± 0.186	0.00	21.67 ± 1.57
TN6	1.96 ± 0.080	8.93 ± 0.493	16.25 ± 2.04	0.00
TN7	42.01 ± 2.54	41.71 ± 6.88	45.00 ± 3.06	4.17 ± 0.393
TN8	47.98 ± 3.98	42.05 ± 4.37	47.50 ± 2.04	38.33 ± 5.96
TN9	57.99 ± 4.98	12.50 ± 0.892	23.75 ± 1.02	15.83 ± 1.44
TN10	37.05 ± 4.14	56.95 ± 3.15	71.67 ± 3.12	0.00
TN11	62.03 ± 3.36	63.81 ± 4.38	75.00 ± 4.25	52.50 ± 6.29
TN12	63.96 ± 2.88	49.81 ± 2.69	76.67 ± 4.38	36.67 ± 2.33
TN13	10.01 ± 1.02	5.90 ± 0.224	0.00	0.00
TN14	59.00 ± 3.71	53.57 ± 2.92	74.17 ± 2.36	0.00
TN15	6.98 ± 0.322	5.36 ± 0.148	0.00	0.00
TN16	29.03 ± 3.06	32.62 ± 2.36	32.50 ± 2.04	0.00
TN17	57.96 ± 2.96	46.14 ± 3.27	77.50 ± 4.78	15.00 ± 1.02
TN18	13.01 ± 1.09	37.14 ± 5.31	75.00 ± 3.88	0.00
TN19	63.96 ± 2.88	53.57 ± 3.79	78.75 ± 8.66	44.17 ± 2.85
TN20	50.98 ± 3.96	16.07 ± 0.835	0.00	0.00
TN21	5.98 ± 0.072	4.52 ± 0.124	67.50 ± 5.10	7.50 ± 0.282
TN22	16.04 ± 1.03	23.21 ± 1.86	31.25 ± 2.04	11.67 ± 0.632
TN23	10.01 ± 0.672	20.21 ± 2.77	60.83 ± 5.62	0.00
TN24	51.99 ± 2.56	22.43 ± 1.59	65.42 ± 2.36	12.50 ± 0.552
TN25	36.99 ± 2.63	21.43 ± 1.08	10.00 ± 1.32	0.00
TN26	7.01 ± 0.470	43.10 ± 2.63	0.00	10.83 ± 0.447
TN27	35.23 ± 2.71	45.81 ± 3.70	72.50 ± 6.42	44.17 ± 3.88
TN28	48.93 ± 2.98	46.14 ± 3.27	0.00	25.00 ± 1.65
TN29	1.99 ± 0.090	42.38 ± 2.36	3.75 ± 0.18	10.00 ± 0.435
TN30	3.95 ± 0.230	42.43 ± 3.45	70.00 ± 4.08	10.00 ± 0.242
TN31	46.94 ± 5.64	37.50 ± 2.69	45.00 ± 2.04	0.83 ± 0
TN32	6.98 ± 0.19	46.43 ± 2.92	75.00 ± 6.62	0.00
TN33	5.02 ± 0.185	42.38 ± 2.36	0.00	12.50 ± 0.752
TN34	37.82 ± 2.88	47.10 ± 3.76	65.83 ± 3.12	0.00
TN35	6.98 ± 0.183	50.81 ± 3.95	76.67 ± 5.32	11.67 ± 0.668
TN36	7.01 ± 0.292	8.93 ± 0.672	0.00	8.33 ± 0.244
TN37	7.01 ± 0.578	32.62 ± 2.36	0.00	8.33 ± 0.435
TN38	53.00 ± 4.24	46.14 ± 3.27	66.67 ± 5.65	24.17 ± 1.18
TN39	56.00 ± 3.71	47.00 ± 4.24	0.00	11.67 ± 0.662
TN40	6.98 ± 0.468	32.14 ± 2.92	0.00	10.83 ± 0.543
TN41	4.99 ± 0.178	11.67 ± 0.578	70.00 ± 2.88	0.00
TN42	8.02 ± 0.541	12.50 ± 0.449	0.00	12.50 ± 0.652
TN43	11.02 ± 0.672	9.29 ± 0.326	0.00	5.83 ± 0.432
TN44	6.98 ± 0.135	8.93 ± 0.552	0.00	20.83 ± 1.88
TN45	6.00 ± 0.224	10.71 ± 0.637	77.50 ± 7.72	14.17 ± 0.542
TN46	57.99 ± 2.54	41.07 ± 2.89	73.33 ± 5.36	0.00
TN47	8.02 ± 0.376	8.93 ± 0.472	38.75 ± 2.76	0.00
TN48	57.99 ± 2.54	42.71 ± 2.74	70.83 ± 7.32	17.50 ± 1.12
TN49	59.98 ± 3.92	50.19 ± 2.69	81.67 ± 9.12	0.00
TN50	10.99 ± 1.08	5.36 ± 0.154	67.50 ± 1.98	0.00
TN51	1.96 ± 0.073	8.93 ± 0.438	0.00	0.00
TN52	6.00 ± 0.080	1.79 ± 0.052	0.00	0.00
TN53	2.97 ± 0.240	12.50 ± 0.472	40.00 ± 2.66	10.83 ± 0.242
TN54	44.98 ± 1.92	46.76 ± 3.33	74.17 ± 4.65	0.00
TN55	60.01 ± 2.84	48.67 ± 5.79	75.83 ± 8.44	5.00 ± 0.680
TN56	57.96 ± 2.96	47.14 ± 2.10	82.50 ± 6.35	0.00
TN57	1.96 ± 0.177	8.93 ± 0.558	64.17 ± 5.42	3.33 ± 0.182
TN58	3.98 ± 0.134	12.50 ± 0.738	6.25 ± 0.252	0.00
TN59	4.96 ± 0.273	16.07 ± 1.19	67.50 ± 3.44	5.00 ± 0.152
TN60	6.00 ± 0.082	8.93 ± 0.662	65.00 ± 5.88	12.50 ± 0.822
TN61	7.93 ± 0.495	5.36 ± 0.293	40.00 ± 3.77	10.00 ± 0.680
TN62	56.16 ± 3.52	8.93 ± 0.548	47.50 ± 4.29	0.00
TN63	7.96 ± 0.269	8.93 ± 0.437	0.00	9.17 ± 0.393
TN64	9.95 ± 0.358	23.21 ± 1.79	67.50 ± 5.32	0.00
TN66	63.09 ± 3.96	55.76 ± 3.51	79.17 ± 7.98	14.17 ± 1.35

sis is less than 300 bp in the study (Davelos et al., 2004). In our earlier study with soils of Meghalaya, India the diversity of *Streptomyces* spp. is higher representing 26 different species (Singh et al., 2022).

Streptomyces bohaiensis is the most abundant (8 isolates) among the 45 *Streptomyces* spp. isolates found in the rhizosphere of rice, okra, brinjal and chilli and in bulk soil, while *S. daghestanicus* is the second most abundant isolate (5 isolates). A study earlier has shown the presence of *Streptomyces* spp. in 20 different plant communities (Adil et al., 2017). In the present study, we have isolated *Streptomyces* spp. from 34 samples among the 40, representing rhizosphere of different crops like rice, maize, sorghum, groundnut,

sunflower, castor, brinjal, okra and chilli, bulk soils of coconut, jatropha and palm fields, thus confirming their ubiquity in soils. Cultural characterization on different ISP media is a routine study involved in the characterization of actinomycetes. In our study, cultural characterization on four different ISP media has shown immense diversity among the soil actinomycetes. Isolates belonging to the same species gave different characterizations among themselves reiterating the fact that there are strain level differences. More than twenty strains showed pigment production in various ISP media used. Earlier many researchers reported similar pigmentation by different actinomycetes strains (Fernandes et al., 2021; Amsaveni et al., 2015).

Antifungal activity of actinomycetes especially, *Streptomyces* spp. are reported in various studies (Rey and Dumas, 2017; Tamreihao et al., 2018; Marimuthu et al., 2020; Sholkamy et al., 2020; Gebily et al., 2021). They have been found to protect a variety of plants from soil-borne fungal diseases to varying degrees. Similarly, the members of the genus *Nocardiopsis* are known for their antifungal activity against different phytopathogens (Bennur et al., 2016). The inherent problems of resistance development by the target fungi and residue in the environment, associated with disease control measures by chemical fungicides led to the search for alternative plant protection measures. Due to the lack of novel antimicrobial metabolites, more and more researchers are focusing their efforts on different ecosystems. In the present study, antifungal activity assay against four fungal pathogens has shown that all 65 actinomycetes strains showed antifungal activity against both *F. udum* and *F. oxysporum* f. sp. *ciceris*. This is consistent with the results of Amini et al. 2016 who reported all 112 actinobacteria isolated as antifungal against *F. oxysporum* f.sp. *ciceris*. In the current study, 74.25 % strain showed antifungal activity against *M. phaseolina* and 60.60 % against *S. rolfsii.* Singh et al. (2016) tested the antifungal properties of 80 actinomycetes strains against *Rhizoctonia solani, Fusarium solani, Macrophomina phaseolina, Sclerotium rolfsii, and Colletotrichum truncatum*, and found various level of antifungal properties of different strains**.** Kamara and Gangwar (2015) isolated 100 actinomycetes strains from 30 rhizospheric soil samples of *Catharanthus roseus* and *Withania somnifera* from different locations of Ludhiana, India, and tested their antifungal activity against viz: *Sclerotium rolfsii*, *Rhizoctonia solani*, *Helminthosporium oryzae*, *Macrophomina phaseolina*, *Penicillium* sp., *Fusarium oxysporum* and *Alternaria alternata*. These findings along with our results suggest that rhizospheric soils have the prospective to identify actinomycetes with potent antifungal activity. To the best of our understanding, the present study offers for the first time a prelude about the unexplored streptomycetes and *Nocardiposis* diversity associated with different crop root rhizosphere and bulk soil from different locations in Tamil Nadu, India.

5. Conclusion

Our findings suggest that there exists a higher level of diversity among the members of the genera *Streptomyces* and *Nocardiopsis* in agricultural fields of Tamil Nadu although the latter is less diverse as compared to *Streptomyces* spp. The study also demonstrates that members of these genera inhabiting the rhizosphere and bulk soils of the study area possess great potential as antifungal agents. Their utilities as biocontrol agents are to be explored in further studies.

Authors contributions

PT, MTZ and SCK performed isolation, morphological characterization, and antifungal activity assay. WAA performed the phylogenetic analysis and compiled the data. MK planned and supervised the experiments and prepared the first draft. HC and AKS collected samples, planned experiments, and improved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102619.

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