



Evaluation of Antagonistic and Plant Growth Promoting Potential of *Streptomyces* sp. TT3 Isolated from Tea (*Camellia sinensis*) Rhizosphere Soil

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Abstract

The present study investigated the antagonistic and plant growth promoting (PGP) potential of actinobacteria TT3 isolated from tea rhizosphere soil of Tocklai tea germplasm preservation plot, Jorhat, Assam, India. It is a Gram-positive, filamentous with flexible spore chains actinobacteria. The 16S rRNA gene sequencing and phylogenetic analysis indicated that TT3 is closely related to genus *Streptomyces* for which it was referred to as *Streptomyces* sp. TT3. It showed very promising PGP traits such as phosphate solubilization, production of indole acetic acid (IAA), siderophore, and ammonia. Evaluation of ethyl acetate extract of TT3 exhibited broad spectrum antagonistic activity against various fungal pathogens. This antagonistic *Streptomyces* sp. TT3 showed positive for polyketide synthase type II (PKS-II) gene, which was predicted to be involved in the production of actinorhodin as a secondary metabolite pathway product using DoBISCUIT database. Further, the crude ethyl acetate extract of TT3 was analyzed by using GC–MS and revealed the presence of significant chemical constituents responsible for antimicrobial activity. Thus, the present study suggests that actinobacteria isolated from the rhizosphere soil may be explored for the production of bioactive compounds and use as a potential candidate for tea and other agricultural application.

Introduction

Microbes are considered as one of the dynamic components in every ecosystem. Microorganisms present in the soil are considered as the key engineers for the functioning of the soil ecosystem and soil restoration process [1]. Moreover, microorganisms in the soil are one of the most important determining factors for soil character and plant productivity. The rhizosphere soil of different agricultural crops was studied for their indigenous microbes. The rhizosphere soil region closely associated with the plant roots is home for diverse plant growth promoting rhizobacteria (PGPR). The application of PGPRs in the agricultural fields in the form of

biofertilizer and biocontrol agents is considered as an alternative to perilous agro-chemicals. In the recent time, PGPRs are widely accepted to use as biofertilizer and biocontrol agents and become a potent biological source. The advancement in the field of PGPR has been progressed because the role of rhizosphere and mechanisms of action of PGPR have got importance in the functioning of our ecosystem [2].

There is a large diversity of biotechnologically important bacterial strains available and actinobacteria is one of them. The phylum actinobacteria are important for producing antibiotic, diverse secondary metabolites, and plant growth promoting (PGP) traits [3]. In addition, it has high efficiency in decomposition and their diversity richness in our environment make them potential biological agents for agriculture and environmental biotechnological application. Actinobacteria produce a wide spectrum of PGP traits or chemical stimulators or modulators which help in plant growth and protection by eliminating other harmful pathogenic microbes. There are several reports on soil dwelling actinobacteria that protects the plant by preventing the growth of different plant pathogens via their antimicrobial metabolites production [4]. Moreover, actinobacteria produce other beneficial PGP attributes such as different plant

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growth hormones, nitrogen fixation, phosphate solubilization, volatile organic compounds, and other plant growth stimulators which make them biotechnologically valuable taxa for agricultural perspective [5, 6].

There is a necessity to study the tea rhizosphere soil in Northeast India because tea is one of the most economically important crops in this region. The economy of our state is significantly dependent upon tea production. Moreover, India has a high reputation in terms of tea production and export of commercial tea and the country contribute 28% of world tea production [7]. However, tea plantations are largely affected by the diverse fungal pathogens. There are 380 fungal pathogens worldwide out of which 190 fungal pathogens have been reported from Northeast India [8]. The tea plantations of Northeast India offer a suitable environment for a large number of fungal diseases and pest invasion which is responsible for the considerable amount of crop loss annually. As a result, tea industry is entirely dependent upon the agro-chemical inputs to encounter with these problems. However, the extensive application of chemicals as fungicides, pesticides, and fertilizers sequentially deteriorated the soil fertility, quality tea production, and our environment as well [9, 10]. Therefore, the present study was carried out to characterize and establish a biotechnologically important strain of actinobacteria for the future application in tea fields. The efficiency of this elite strain was evaluated through the production of antimicrobial metabolites and PGP activity. This study was also investigated the mode of action of the strain for antimicrobial activity by targeting the polyketide synthase gene. Further, GC-MS profiling of the TT3 metabolites was studied to identify the major chemical constituents for antimicrobial activity.

Materials and Methods

Isolation and Preservation of TT3

The tea rhizosphere soil samples with the roots from 5–30 cm depth located at Tocklai tea germplasm preservation plot, Tocklai Tea Research Institute, Jorhat, Assam, India (26° 45' 18.40" N 94° 13' 16.92" E) were collected during March, 2013. Actinobacteria were isolated using Actinomycetes isolation agar and Streptomycetes agar media (HiMedia, India) amended with rifampicin (2.5 µg/mL) and amphotericin B (75 µg/mL). The isolation media plates were incubated at 28 °C for 7–10 days. The TT3 isolate was obtained from the isolation media plate of Actinomycetes isolation agar. The colony of TT3 isolate was sub-cultured several times to get the pure culture on GLM agar medium (Yeast extract, 3 g/L; malt extract, 3 g/L; peptone Type I, 5 g/L; starch, 10 g; agar, 20 g; pH 7.4). Further, it was

preserved on slants at 4 °C and 15% glycerol stock at –80 °C for a longer period.

Morphological and Molecular Characterization of TT3

For the study of morphology and sporulation, the culture of TT3 was grown on GLM agar media with the inclination at 45° angle cover slip technique and incubated for 7 days at 28 °C. The spore chain morphology of TT3 was observed under light microscope and scanning electron microscope. For the scanning electron microscope analysis, the TT3 grown cover slip was mounted on a metal stub and then the stub was coated under vacuum with a gold film. After coating, the specimen was examined under the field emission scanning electron microscope (FE-SEM) (Zeiss Sigma VP, Germany). The field was first scanned at lower magnification to detect the line of growth and then a higher magnification (up to 10 KX) was selected to examine the clear, intact sporulating structures of TT3.

For molecular characterization, genomic DNA of the TT3 was extracted by using nucleopore gDNA fungal bacterial mini kit (Genetix, India) according to the manufacturer's instructions and the DNA was analyzed by 0.8% agarose gel electrophoresis. Then the PCR amplification of 16S rRNA gene was carried out by using primers PA (5'-AGAGTTTGA TCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTT ACGACTT-3'). The PCR reaction was performed in thermocycler (Proflex PCR system, Applied Biosystems, USA) programmed with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 30 s at 72 °C with a final extension of 72 °C for 2 min. The amplified product of 16 s rRNA gene was electrophoresed on a 1.8% agarose gel with ethidium bromide in 1 × TAE buffer at 50 V for 45 min. PCR product was visualized under ChemiDoc (BioRad, USA). Further, the PCR product was purified for sequencing by GenElute PCR clean up kit (Sigma-Aldrich, USA) and delivered to First BASE Laboratories, Malaysia. The raw forward and reverse sequences of TT3 were analyzed to filter the low-quality base calls by using Sequence Scanner 2.0 software (Applied Biosystems). The low-quality base calls from the both sequences were trimmed and aligned to remove the overlap regions to generate contigs. Then the contigs were assembled to one complete sequence and checked for the presence of chimera using DECIPHER software [11]. The assembled sequence of TT3 strain was verified and compared against nucleotide databases using NCBI BLASTn and EzTaxon server 2.1 programs to designate the taxonomic status [12].

Phylogenetic Analysis

For the phylogenetic analysis, the identified 16S rRNA gene sequence of TT3 aligned with their closest homology sequences using multiple sequence alignment program CLUSTAL W executed in MEGA 6 software by using default parameters [13]. The reference sequences were collected from the GenBank database. Pairwise evolutionary distances were computed with the help of Kimura's 2 parameter model [14]. The phylogenetic tree was constructed by neighbor-joining (NJ) method using MEGA 6 program and the robustness of the tree was estimated by 1000 bootstrap replications of the original dataset using p-distance model [15]. The partial 16S rRNA gene sequence of TT3 was deposited in the GenBank under the accession no. KT892738.

Screening for In Vitro Plant Growth Promoting (PGP) Traits

Phosphate Solubilization

The phosphate solubilization was initially carried out on Pikovskaya's agar media. Further, the estimation of phosphate solubilization was performed in Pikovskaya's liquid medium embedded with tri-calcium phosphate as described by Fiske and Subbarow [16] with slight modification using ammonium molybdate reagent. The absorbance was measured at 650 nm in three replicates by multimode reader (Varioskan flash, Thermo Scientific, USA). The quantification of phosphate solubilization was calculated by using the calibration curve prepared with KH_2PO_4 .

IAA Production

The IAA production was carried out by inoculation of TT3 in M9 glucose minimal salts media [17]. The media is supplemented with 0.2 μm membrane filter-sterilized L-tryptophan solution at 100 $\mu\text{g}/\text{mL}$ concentration. The TT3 culture was grown for 72 h and harvested by centrifugation (12,000 rpm for 10 min). For quantification of IAA production, the supernatant from the culture was mixed with the Salkowski's reagent (48 mL 35% HClO_4 containing 2 mL 0.5 M FeCl_3) in 1:2 ratio (bacterial supernatant: reagent) at room temperature (RT). After incubation for 25 min at RT, the IAA was estimated at 530 nm in 96-well microtiter plates by multimode reader (Varioskan flash, Thermo Scientific, USA) with three replicates [18]. The quantification of IAA produced by TT3 was calculated using the standard curve prepared by commercial IAA at different concentrations (Sigma, Aldrich, USA).

Siderophore Production

Siderophore production of TT3 was carried out on Chrome Azurol S (CAS) agar medium [19]. The siderophore production was observed by the formation of orange halos around the bacterial colonies due to the formation of ternary complex chrome azurol S/Fe(III)/hexadecyltrimethylammonium bromide. The estimation of siderophore production was carried out as described by Patel et al. [20]. For the quantification, the 0.5 mL of culture supernatant was mixed with 0.5 mL of CAS reagent and the uninoculated one served as reference. The experiment was performed with three replicates. The absorbance was read in the multimode reader (Varioskan flash, Thermo Scientific, USA) at 630 nm. Siderophore content in the sample was calculated by using the following formula:

$$\% \text{ siderophore units} = \text{Ar} - \text{As}/\text{Ar} \times 100$$

where Ar and As are absorbance of reference (CAS reagent) and absorbance of sample at 630 nm.

Ammonia Production

The ammonia production of TT3 was carried out using peptone water broth media. The culture of TT3 was inoculated in the peptone media for 48 to 72 h at 30 °C. After incubation, the culture supernatant was obtained by centrifugation and mixed with Nessler's reagent. The presence of ammonia was indicated by the formation of brown to yellow color in the solution. The presence of ammonia was estimated by taking the absorbance at 450 nm using the multimode reader (Varioskan flash, Thermo Scientific, USA) with three replicates. Ammonia produced by TT3 was quantified using standard curve of ammonium sulfate [21].

In Vitro Antagonistic Bioassay

Test Fungal Pathogens

The six fungal pathogens were used for the study i.e., *Pestalotiopsis theae* (ITCC 6599), *Curvularia eragrostidis* (ITCC 6429), *Glomerella cingulata* (MTCC 2033), *Rhizoctonia solani* (MTCC 4633), *Fusarium oxysporum* (MTCC 284), and *Nigrospora sphaerica* (KJ767520). The fungal pathogens were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India and Indian Type Culture Collection (ITCC); Indian Agricultural Research Institute, New Delhi, India. The tea fungal pathogen *Nigrospora sphaerica* was isolated, characterized, and preserved at

Institute of Advanced Study in Science & Technology, Guwahati, India. The fungal pathogens were cultured in PDA medium at 25 °C.

The antagonistic activity against the selected fungal pathogens was estimated by comparing the diameter of fungal mycelium on control and test plates. The percentage of inhibition was calculated by using the formula $C - T/C \times 100$, where C and T are the fungal mycelial diameter on control and the test plate, respectively [22].

Spot Inoculation Method

The TT3 was subjected to evaluate for preliminary antagonistic activity against *P. theae*, *R. solnai*, *F. oxysporum*, and *N. sphaerica* on PDA agar medium. For spot inoculation method, GLM broth culture of TT3 was adjusted to 1×10^8 CFU/mL and spot inoculated at one edge on the PDA plates. Then the 5 mm agar plug of each test fungal mycelium grown on the PDA plate was placed at the other edge of the plate. The plates were incubated at 28 °C for 3–5 days. The control plates were prepared with the fungal agar plug without inoculation of TT3 culture.

Disk Diffusion Method by Ethyl Acetate Extract of TT3 (EA-TT3)

The antagonistic activity of TT3 against six selected fungal pathogens i.e., *P. theae*, *C. eragrostidis*, *G. cingulata*, *R. solani*, *F. oxysporum*, and *N. sphaerica* was further evaluated by EA-TT3 using disk diffusion method on PDA agar medium plates with three replicates [23]. For this experiment, TT3 was grown in submerged culture in 250 mL flasks containing 100 mL of GLM liquid medium. The 5 days old culture of TT3 grown on GLM agar media was inoculated in the liquid medium flasks. The culture was grown in a rotary shaker at 200 rpm and 28 °C for 7 days. After incubation, broth culture media was separated from the mycelium by centrifugation at 7000 rpm for 15 min. Crude extract of TT3 was obtained from the culture filtrate by solvent extraction using ethyl acetate in 1:1 ratio (v/v). The ethyl acetate extract of TT3 (EA-TT3) was finally recovered by evaporation of ethyl acetate using rotary evaporator. The concentrated extract obtained was then dissolved in 10% dimethyl sulphoxide (DMSO) at a concentration of 1 mg/mL prior to antifungal bioassay. The 20 µL of the EA-TT3 was loaded into 6 mm diameter of sterile disk placed on the PDA plates spread with fungal test pathogens. The 20 µL of 10% DMSO loaded on sterile disk was served as negative controls. The antifungal activity was observed after 3–5 days of incubation at 25 °C.

PCR-Based Detection and Analysis of Biosynthetic Genes (PKS-I, PKS-II and NRPS)

The PCR-based detection of biosynthetic genes was amplified by using degenerate primers. The K1F (5'-TSAAGTC-SAACATCGGBCA-3') and M6R (5'-CGCAGGTTS CSGTACCAGTA-3') were used for amplification of PKS-I ketosynthase and methylmalonyl transferase domain sequences; KSαF (5'-TSGCSTGCTTGGAYGCSATC-3' and KSαR (5'-TGGAANCCGCCGAABCCGCT-3') were used for PKS-II genes; A3F (5'-GCSTACSYSATSTACACSTC-SGG-3') and A7R (5'-SASGTCVCCSGTSCGGTAS-3') were used for amplifications specific for NRPS adenylation domain sequences [24]. For the PCR reaction, 10 µL of PCR cocktail contained 1 µL of 10×Taq DNA buffer, 2.5 mM dNTP mix, 0.2 µM of primers, 1U Taq polymerase, and 1 µL of 10 ng concentration of template DNA. Touchdown PCR was carried out for the amplification of PKS-I genes to increase the specificity. For that, annealing temperature was set 10 °C above the expected annealing temperature of 56.8 °C and was decreased by 1 °C every second cycle until a touchdown of 46.8 °C. The thermal cycling conditions for the amplification of PKS-II and NRPS genes were programmed as follows: initial denaturation at 94 °C for 5 min; followed by 35 cycles at 94 °C for 1 min, 65 °C (PKS-II) and 63 °C (NRPS) for 1 min, 72 °C for 2 min, and final extension at 72 °C for 10 min.

For analysis of biosynthetic genes, the nucleotide sequences were translated to protein sequences using the ORF-Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). The inferred amino acid sequences of the respective biosynthetic genes were used as queries to search the related proteins in the NCBI nr protein database using the BLASTP algorithm with the default parameters. Further, gene clusters involved in the synthesizing of the secondary metabolites derived from bacteria were identified by BLAST homology search using the DoBISCUIT database which is a secondary metabolite biosynthetic gene clusters database [25].

GC–MS Analysis of EA-TT3

The identification of the signature chemical constituents present in EA-TT3 was analyzed using GC–MS as described earlier with some modifications [26]. For the GC–MS analysis, sample was dissolved in HPLC-grade methanol and filtered through 0.2 µm filter. GC–MS analysis was performed on a Shimadzu GC 2010 plus-triple quadrupole (TP-8030) and GC–MS/MS fitted with EB-5 MS column (length-30 m, thickness-0.25 µm, ID-25 mm). The oven programme started at 40 °C for 5 min, ramped at 10 °C/min to 280 °C for 10 min, again ramped to 290 °C at 5 °C/min, and finally held for 10 min. Sample was injected at 300 °C using helium as carrier gas (1 mL/min), split at the ratio of

20:1. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV with a continuous scan from 45 to 600 m/z. The peaks were identified by matching the mass spectra with the National Institute of Standards and Technology (NIST, USA) library.

Data Analysis

All experiments were performed in triplicates to calculate the mean values and data were expressed as mean \pm standard deviation. Bioinformatics analysis was carried out by using specific bioinformatics tools.

Results

Morphological and Molecular Characterization of TT3

Actinobacterial strain TT3 was isolated from the soil of tea rhizosphere located in Tocklai tea Germplasm Preservation plot, Tocklai Tea Research Institute. It was aerobic, Gram-positive, and morphologically filamentous in nature. Further, TT3 grown on GLM agar media for 7 days appeared as smooth spore chain morphology with profuse growth of both aerial and vegetative hyphae, which were well developed and not fragmented. The vegetative hyphae were observed as light brown in color, while aerial mycelia were white in color on GLM agar media. Moreover, observation of SEM image of TT3 revealed the formation of long aerial mycelia and spore chains are flexible with smooth surface (Table 1, Fig. S1).

The 16S rRNA gene sequence of TT3 (1371 bp) was analyzed and submitted to NCBI GenBank database under the accession number KT892738. The 16S rRNA gene sequence of TT3 showed highest similarities with *Streptomyces kronopolitis* NEAU-ML8 (KP050495) (99.7%), *S. chattanooensis* KPP02992 (99.6%) (KM573812), and *S. lydicus* strain A2 (99.4%) (KF712383). As there are more than one species at the same similarity score (> 99.5%), threshold, and *E*-value, an ambiguity was created in the strain identification. This is also reflected in the phylogenetic tree constructed based on Neighbor-joining method, which showed separate distinct clusters together with their closest known taxa (Fig. 1). However, the morphological and genomic data

of TT3 indicated that the strain TT3 represented the genus *Streptomyces* for which it was referred to as *Streptomyces* sp. TT3.

Multifarious Plant Growth Promoting (PGP) Traits Production

Screening for Phosphate Solubilization and Production of IAA, Siderophore, and Ammonia

The *Streptomyces* sp. TT3 showed positive result for phosphate solubilization, and production of IAA, siderophore, and ammonia. The quantitative estimation of phosphate solubilization, IAA, siderophore, and ammonia production showed 187 $\mu\text{g}/\text{mL}$, 34.6 $\mu\text{g}/\text{mL}$, 38.6% and 5.4 $\mu\text{mol}/\text{mL}$, respectively (Table 2).

In Vitro Antagonistic Bioassay

Streptomyces sp. TT3 showed preliminary antagonistic activity against *N. sphaerica*, *P. theae*, *R. solnai*, and *F. oxysporum* (Fig. S2). Furthermore, ethyl acetate extracts of TT3 (EA-TT3) showed promising antagonistic activity against all the six selected fungal pathogens i.e., *P. theae*, *C. eragrostidis*, *G. cingulata*, *R. solnai*, *F. oxysporum* and *N. sphaerica* (Table 2, Fig. S3).

Detection and Analysis of Biosynthetic Genes in TT3

The PKS-I, PKS-II, and NRPS biosynthetic genes were screened by degenerate primers. However, *Streptomyces* sp. TT3 showed only the presence of PKS-II gene. The PCR amplification of PKS-II gene using degenerate primers resulted in amplicon size of 600–700 bp confirming the presence of PKS-II gene. The gene was sequenced and translated to amino acid sequence for similarity search of their related proteins in NCBI database using GenBank BLASTP. The secondary metabolite pathway products were also identified using DoBISCUIT database. It showed that PKS-II gene derived from *Streptomyces* sp. TT3 shared 74.24% similarity to β -ketoacyl synthase/acyl transferase of *Streptomyces coelicolor* A3(2) (GenBank accession no. CAA45043) at amino acid level. The detection of PKS-II pathway product of TT3 was predicted to be actinorhodin which is a benzoisochromanone quinone class of antibiotic.

Table 1 Morphological characterization of strain TT3

Strain ID	Origin	Aerial mycelia color	Substrate mycelia color	Diffusible pigment	Spore surface	Spore chain morphology
TT3	Tocklai tea germplasm preservation plot, Jorhat, Assam, India	White	Brown	–	Smooth	Flexible

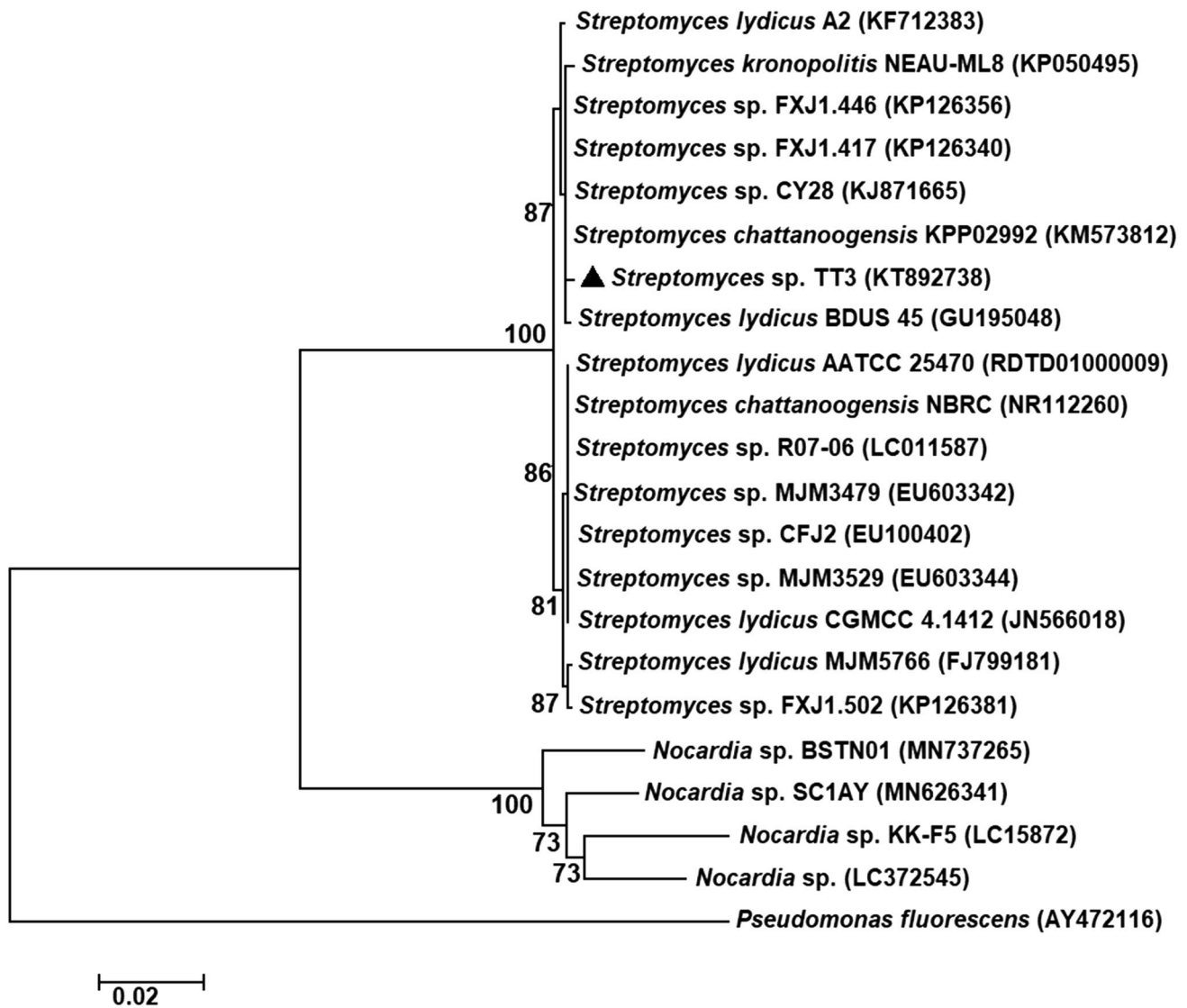


Fig. 1 NJ-phylogenetic tree showing the evolutionary relationship between *Streptomyces sp.* TT3 and the selected reference strains from GenBank database. The bar represents 0.02 substitutions per site, bootstrap values ($n = 1000$) are displayed

Table 2 Screening of *Streptomyces sp.* TT3 strain for in vitro plant growth promoting traits and antagonistic activity against fungal pathogens

Strain code	Gram reaction	PGP traits				
		IAA production ^a ($\mu\text{g/mL}$)	Phosphate solubilization ^b ($\mu\text{g/mL}$)	Ammonia production ^c ($\mu\text{mol/mL}$)	Siderophore production ^d (%)	
TT3	+	34.6 ± 0.1	187 ± 1.8	5.4 ± 0.1	38.6 ± 1.4	
Strain code	<i>Nigrospora sphaerica</i>	<i>Pestalotiopsis theae</i>	<i>Curvularia eragrostidis</i>	<i>Glomerella cingulata</i>	<i>Rhizoctonia solani</i>	<i>Fusarium oxysporum</i>
Antagonistic activity						
Growth inhibition (%) [*]						
TT3	48.6 ± 0.1	38.6 ± 0.3	36.6 ± 0.1	34.6 ± 0.3	32 ± 0.1	45.2 ± 0.3

^{a,b,c,d} Values are mean of three replicates \pm SD

^{*} Values are mean of three replicates \pm SD

GC–MS Analysis

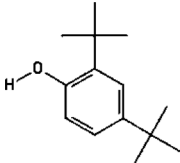
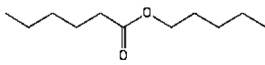
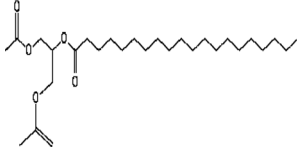
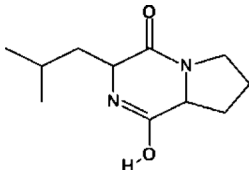
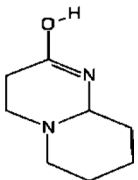
The spectrum of chemical compounds produced by *Streptomyces* sp. TT3 was detected by using GC–MS. Five major chemical compounds i.e., Phenol, 2,4-bis(1,1-dimethylethyl)- Hexanoic acid, pentyl ester; Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester; Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl); and Octahydro-2H-pyrido(1,2-a) pyrimidin-2-one were identified based on their retention time, peak area, molecular weight, and molecular formula through comparison of their mass spectra with the NIST library. The retention time, area percentage, molecular formula, molecular weight, and chemical structures of these five compounds were illustrated (Table 3, Fig. S4).

Discussion

The microbes present in the soil are crucial for the soil ecosystem functioning and they are well established for their ability to production of diverse secondary metabolites. However, rhizosphere soil is one of the most

important niches of soil for intense plant–microbe interaction. Rhizosphere soil is home to diverse microorganisms where actinobacteria microbial group is also found. Actinobacteria are well studied for their board spectrum of secondary metabolites with antimicrobial properties that suppress the growth of diverse pathogens including human and plants [4, 27]. Actinobacteria isolated from different habitats are investigated for their prospective of wide array of natural bioactive compounds production [28]. However, reports on actinobacteria isolated from tea rhizosphere soil are inadequate. Therefore, we have carried out the research on tea rhizosphere soil of Northeast India for isolation and characterization of diverse PGPRs with their functions. During our study, we have found the actinobacterial strain TT3 to be having the board spectrum of antifungal activity against different tea foliar pathogens including our isolated tea foliar fungal pathogen *N. sphaerica*. The sequencing and analysis of 16S rRNA gene of TT3 shared highest similarities with *Streptomyces* genus such as *S. kronopolitis* NEAU-ML8 (KP050495) (99.7%), *S. chatta-noogensis* KPP02992 (99.6%) (KM573812), and *S. lydicus* A2 (99.4%) (KF712383), and thus, in this study the strain TT3 is designated as *Streptomyces* sp. TT3.

Table 3 Major chemical constituents identified from EA-TT3 extract through Gas chromatography–mass spectrometry (GC–MS)

Compound name	Retention time	Area (%)	Formula	Molecular weight (g/mol)	Chemical structure	Activity	References
Phenol, 2,4-bis(1,1-dimethylethyl)	19.76	15.43	C ₁₄ H ₂₂ O	206		Antimicrobial activity	[45, 46]
Hexanoic acid, pentyl ester	22.53	8.17	C ₁₁ H ₂₂ O ₂	186		No antimicrobial activity reported	–
Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester	23.27	4.50	C ₂₇ H ₅₀ O ₆	470		Antimicrobial Activity	[47]
Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	24.79	11.85	C ₁₁ H ₁₈ N ₂ O ₂	210		Antimicrobial Activity	[26, 48, 49]
Octahydro-2H-pyrido(1,2-a) pyrimidin-2-one	28.89	6.55	C ₈ H ₁₄ N ₂ O	154		No antimicrobial activity reported	–

Promising PGP activity added more value to the *Streptomyces* sp. TT3. It showed phosphate solubilization and production of IAA, siderophore, and ammonia. Actinobacteria are reported for different PGP traits such as production of various plant growth hormones including auxins, gibberellins, and cytokinin, siderophores, phosphate solubilization, and nitrogen fixation etc. [5, 6]. Though *Streptomyces* was studied for the production of antibiotics, but a significant effort has been also devoted to monitoring the populations of potential biocontrol *Streptomyces* species for agricultural application. In our study, *Streptomyces* sp. TT3 showed promising PGP activity such as production of IAA, siderophore, and ammonia along with phosphate solubilization which are important traits for the plant growth and proved its potential to be considered as a PGP strain.

The *Streptomyces* sp. TT3 exhibited potential antagonistic activity against the tested fungal pathogens. Further, ethyl acetate extraction of TT3 i.e., EA-TT3 crude extract consisting of a mixture of metabolites significantly suppressed the growth of fungal pathogens. Similarly, *S. lydicus* WYEC108 strain has been previously characterized and reported for the biocontrol of fungal root and seed rots [29]. Soil bacteria that reside in the rhizosphere soil restrain the growth of several phytopathogens by producing different biocontrol metabolites or antibiotics. These are structurally diverse metabolites having antimicrobial properties against various phytopathogens [30, 31]. Moreover, in vitro productions of antimicrobial or antibiotic compounds are usually assumed to be responsible for in vivo biocontrol agents [32].

Polyketides are biotechnologically promising structurally and chemically diverse secondary metabolites which have different vital biological functions especially antimicrobial properties. Polyketides chains produced by polyketides synthases (PKSs) are derivatized or modified into complex bioactive molecules through sequential catalytic activities. These enzymes facilitate biosynthesis of diverse structurally complex bioactive molecules by combinatorial use of a specific sequential order of catalytic domains [33]. *Streptomyces* spp. are known for the production of polyketides and non-ribosomal polyketide compounds [34]. In the present study, we used PCR primers specific for the detection of NRPS, PKS-I, and PKS-II sequences in actinobacteria. *Streptomyces* sp. TT3 showed positive PCR amplification of the beta-ACP synthase and ketosynthase genes of PKS-II gene. Amplification of PKS-I ketosynthase, methylmalonyl transferase domain sequences, and NRPS adenylation domain sequences is not detected. The type II polyketide synthases (PKS-II) are complexes of discrete enzymes composed of seven separate mono- or bi-functional enzymes, which act in an iterative manner during synthesis. PKS-II are mainly known for biosynthesis of the aromatic polyketide compounds e.g., actinorhodin [35] and tetracenomycin [36, 37]. *Streptomyces venezuelae* strain was also reported for

being associated with the KS α of the jadomycin and ardicin pathways [38]. Also, two new polyketides i.e., actinofuranones A and B were identified from the culture extract of a marine-derived *Streptomyces* strain CNQ766 [39]. In our study, type II PKS gene was observed in *Streptomyces* sp. TT3 which showed excellent antifungal activity. PKS-II gene present in the *Streptomyces* sp. TT3 was predicted by using DoBISCUIT database involved in the production of pathway product actinorhodin which is a benzoisochromane quinone dimer polyketide antibiotic classified under quinone class of compounds [35]. There are diverse groups of antimicrobial molecules produced by PGPRs and among them the polyketides molecules produced by the PGPRs such as 2,4-Diacetyl phloroglucinol, Pyoluteorin, and Mupirocin are highly efficient in the suppression of plant-associated pathogens [40]. Moreover, antifungal activity of *B. amyoliquefaciens* FZB42 was also reported due to the production of bacillomycin D active compound and it suppressed the growth of plant pathogenic fungus *F. oxysporum* [41].

To detect the other potential volatile compounds in the *Streptomyces* sp. TT3, we have performed the GC–MS analysis of EA-TT3 crude extract. The GC–MS is an authoritative analytical technique for the detection of various chemical signatures in crude metabolites and it has been incorporated in different studies [26, 42–44]. We have found five most significant compounds from the EA-TT3 crude extract with different retention time and abundance. There are plentiful of reports available on GC–MS analysis of microbial metabolites. For instance, among the five major compounds detected, Phenol, 2,4-bis(1,1-dimethylethyl) compound was reported from *Pseudomonas fluorescens* TL-1 metabolites by GC–MS analysis for antifungal activity against various fungal phytopathogens [45]. Similarly, purification and mass spectrometric analysis of active compound phenol and 2,4-bis(1,1-dimethylethyl) derived from marine bacteria lead to the inhibition of quorum sensing-mediated biofilm formation in the uropathogen *Serratia marcescens* [46]. Recently, study conducted by the Kumari et al. [47] described the different chemical compounds including the 2,6-di-Tert butyl phenol; Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl ester; Pyrrolo[1,2-a] pyrazine-1,4-dione, and hexahydro-3-(2-methylpropyl) detected by GC–MS analysis from the antimicrobial extract of Actinomycetes AIA6 isolate which were also detected in EA-TT3 extract. Similarly, the Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) derived from microbes was also documented as bioactive compound in several publications. For example, the compound Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) was reported from *Streptomyces mangrovisoli* MUSC 149 extract by GC–MS analysis and they demonstrated the antioxidative properties of the compound which may crucial roles in the prevention and treatment of chronic diseases [26]. Interestingly,

the same compound has been reported from marine *Streptomyces* sp. VITPSA by GC–MS analysis, which possesses protease inhibitor and shows excellent antiretroviral activity [48]. The compound Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) was also isolated and recently reported from marine bacteria *Bacillus tequilensis* MSI45 extract and was characterized by using spectroscopic analysis such as FT-IR, NMR, and GC–MS which effectively controlled the growth of multi-drug resistant *Staphylococcus aureus* [49]. Therefore, these compounds are well recognized and reported for their wide spectrum of antimicrobial activities and thus GC–MS-detected compounds in our EA-TT3 extract could be responsible for the antifungal activity for the different tea fungal phytopathogens. Moreover, it was suggested that the secondary metabolites of actinobacteria are preferred over fungal metabolites because they are less phytotoxic. It was also stated that over 50% of fungal metabolites were phytotoxic, herbicidal, and pesticidal where only 2% of phytotoxic actinobacterial products were phytotoxic [50]. Thus, we assume that these compounds could be the key contributing factor for the antifungal activities of EA-TT3. The results observed in the present research study also suggest that the metabolites extracted from *Streptomyces* sp. TT3 need further validation to prove the efficiency of the metabolites. However, from the present study, it is indicated that this tea rhizosphere-derived *Streptomyces* sp. TT3 has the potential to consider as antifungal and PGP strain and this strain could be safely and efficiently used as an alternative to chemical control in tea field with further investigation in future.

Conclusion

Streptomyces are considered as one of the most prominent genera in the actinobacteria group. Most of the present commercially useful antibiotics were produced by genus *Streptomyces*. However, study and application of this potential species as a biological control is very limited in our agricultural field. Our study revealed the important attributes of the *Streptomyces* sp. TT3 strain and its future potential application in the tea and other agricultural field. The diversity of actinobacteria species is rich in the rhizosphere soil and may be involved in different active metabolites production and PGP functions. The study further suggests the exploration of diverse actinobacteria associated with tea rhizosphere soil for the potential application in our tea and other agricultural systems.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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