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Identification of biostimulant and microbicide compounds from *Streptomyces* sp. UC1A-3 for plant growth promotion and disease control

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Abstract The plant growth promotion and antagonistic potential of *Streptomyces* against phytopathogens was assessed. Total fourteen *Streptomyces* strains were derived from rhizosphere soil of *Capsicum annum* (Chilli) from the agricultural fields in Udhagamandalam, Nilgiris, Tamil Nadu, India. All strains were evaluated for plant growth promoting *in vitro* e.g. production of indole acetic acid, ammonia, siderophores, chitinase, cellulase, protease, amylase and inorganic phosphate solubilisation. In addition, antagonistic activity was also tested against *Ralstonia solanacearum*, *Xandhomonas oryzae*, *Fusarium oxysporum*, *Alternaria* sp., *Macrophomina* sp., and *Magnaporthe oryzae*. Further, bioactive compounds from the strain UC1A-3 was analyzed through gas chromatography–mass spectrometric technique. Three strains showed the highest level to promote plant growth promoting and antagonistic activity especially the strain UC1A-3 revealed maximum level of seed germination and increased shoot and root length in Chilli plants. Totally, twenty-nine compounds were detected, most of which were aromatic compound derivatives. In particular, Phthalic acid ($C_8H_6O_4$), Pentadecanoic acid ($C_{15}H_{30}O_2$), i-Propyl 12-methyltetradecanoate ($C_{18}H_{36}O_2$), l-(+)-Ascorbic acid 2,6-dihexadecanoate ($C_{38}H_{68}O_8$), 1-Nonadecene ($C_{19}H_{38}$), 1-Heptacosanol ($C_{27}H_{56}O$) were reported as antimicrobial properties. Findings of the present study evidenced that *Streptomyces* strain UC1A-3 would be a promising candidate for agricultural crop improvement, since it has showed the potential *in-vitro* plant growth and biocontrol activities against the tested phytopathogens.

Keywords: *Streptomyces*; Bioactive metabolites; Plant growth promotion; Gas chromatography–mass spectrometry; Phthalic acid

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Introduction

Plant growth and disease control is greatest challenge in the field of agriculture (Almeida *et al.*, 2019). Several fungal and bacterial organisms cause severe disease damage to numerous economically important plants at global level (Wang *et al*., 2019). The current practice for disease control relies on the application of synthetic chemicals or the development of resistant varieties (Tripathi *et al*., 2019), leading to cause the pollution, imbalance soil ecology, and the development of resistance among the phytopathogens. Likewise, the resistant crop development is also being disruption of ecosystem and biodiversity, high cost of cultivation and human health risk. These problems can be possible solved bio-based approach for alternative control the various phytopathogens and plant growth promotion (Alori and Babalola, 2018).

Actinobacteria are high in G+C content in their genome. They are the predominant producers of the most commercially available secondary metabolites especially enzyme inhibitors, antioxidants, anti-inflammatory agents, pesticides, and plant-growth promoting substances (Thilagam and Hemalatha, 2019). In addition, actinobacteria e.g. *Streptomyces*, *Thermomonospora* and *Micromonospora* are protected plant diseases causing from wide range of phytopathogens by the induction of antimicrobial metabolites, enzymes, and plant growth promoters (Passari *et al*., 2019). There are very few studies reported on biocontrol and plant growth promotion of actinobacteria in Solanaceae. Hence, *Streptomyces* strains from the rhizosphere soil of *Capsicum annum* (Chilli) were investigated for their plant growth and disease control.

Materials and methods

Sample collection

Rhizosphere soils were collected from *Capsicum annum* (Chilli) agricultural field in Udhagamandalam (Lat. 11º48’N; Long. 76º77’E), The Nilgiris, Tamil Nadu, India. The collected samples were dried for 3 days at room temperature and passed 2 mm sieve to collect coarse waste. Further, the samples were pretreated by dry heat in hot air oven at 55 ℃ for 10 mins, in order to retard the growth of slime forming bacteria and fungi (Radhakrishnan *et al*., 2007).

Isolation and characterization of actinobacteria

Soil plate method was used to isolate the target actinobacteria from soil samples using the medium starch casein nitrate (SCN) agar supplemented with

cycloheximide (25 µg/ml) and nalidixic acid (20 µg/ml) to prevent fungal and bacterial contamination. The isolated plates were incubated at 28 ℃ for one month. During incubation, colonies with distinct actinobacterial morphology developing on SCN agar plates were transferred and purified using International *Streptomyces* Project 2 (ISP2) agar medium by incubating at 28 ℃ for 7-10 days. After the purity confirmation, morphologically dissimilar actinobacterial colonies were sub-cultured on ISP2 agar slants as well as in 20% glycerol broth and stored at 4 ℃ and 20 ℃ in deep freezer. Characteristics of culture e.g. aerial mass color, consistency, growth rate, reverse side pigment and soluble pigment were observed. The microscopic characteristics e.g. aerial and substrate mycelia, fragmentation of mycelia and spore chain were recorded.

Determination of antagonistic potential

Phytopathogens including bacteria *Ralstonia solanacearum*, *Xanthomonas oryzae* and fungal pathogens *Fusarium oxysporum*, *Alternaria* sp., *Macrophomina* sp., and *Magnaporthe oryzae* were obtained from Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu and India. Bacteria cultures were maintained in *Pseudomonas solanacearum* medium and Modified Wakimoto's Agar (MWA) medium and fungi were maintained in potato dextrose agar.

Antagonistic screening against plant bacterial pathogens

The antibacterial activity of *Streptomyces* strains against plant bacterial pathogens was screened by adopting agar well diffusion method (Saadoun and Muhana, 2008). Briefly, the *Streptomyces* strains were cultivated on ISP2 broth and incubated in rotary shaker at 28 ℃ with 250 rpm for 7 days. Cell free supernatant was collected by centrifuge for 5 min at 10,000 rpm to test against bacterial pathogens. The tested pathogen was streaked to nutrient agar plate and sterile cork borer used to create a 5 mm of diameter well. In each well, 50µl of cell free supernatant was added and incubated at 28 ℃ for 24 h. The pathogenic growth suppression was measured and showed as inhibition zone.

Antagonistic screening against plant fungal pathogens

Streptomyces strains were checked for antifungal activity by dual culture method (Bredholdt *et al*., 2007). The fungal pathogen was transferred to the middle of agar plate and *Streptomyces* strains were streaked at the periphery of the agar plates. All plates were incubated at 28 ℃ for 7-14 days.

The mycelial growth was computed and compared with control. The plates growing pathogen alone served as control. The inhibition was calculated as follows:

$$\% \text{ inhibition} = [1-(\text{Fungal growth}/\text{Control growth})] \times 100.$$

In-vitro screening of Streptomyces for plant growth promotion and enzyme production

Indole acetic acid (IAA*)* production

One millilitre aliquot of the spore suspensions (~10^6 CFU ml^{-1}) of *Streptomyces* strains were transferred to 50 ml of Yeast extract-Tryptone (YT) broth. The transferred flasks were incubated on a shaking incubator at 30℃ with 200 rpm for 5 days. The liquid cultures were subjected for centrifugation for 30 min. IAA production was done by mixing 2 ml supernatant and 4 ml Salkowski reagent (1 ml of 0.5 M $FeCl_3$ in 49 ml $HClO_4$ solution (35%, w/v)). The pink in color after 30 min incubation in a darkroom expressed the IAA activity. Optical density was evaluated by spectrophotometer at 530 nm. The amount of IAA was expressed in µg/ml (Passari *et al*., 2015).

Phosphate solubilization

The experiment was conducted in 250 ml Erlenmeyer flasks containing 50 ml of NBRIP broth which consisted of 10 g tricalcium phosphate (TCP), 10 g $MgCl_2.6H_2O$, 5 g $MgSO_4.7H_2O$, 0.25 g KCl and 0.2 g $(NH_4)2SO_4$. A 1 ml aliquot of *Streptomyces* spore suspensions (~10^6 CFU ml^{-1}) was transferred to flasks and incubated for 7 days on a rotary shaker (200 rpm) at 30℃. The supernatant was collected from each strain by centrifugation at the rotation speed of 10,000 rpm for 10 min. The phosphate quantity was performed using ascorbic acid method (Doumbou *et al*., 2001). The absorbance was measured at 880 nm using a spectrophotometer, then compared with the standard curve of KH_2PO_4. The amount of KH_2PO_4 was expressed in mg/100ml.

Siderophore production

The siderophore production by *Streptomyces* strains was investigated by modifying chrome azurol S (CAS) plate assay (Hu and Xu, 2011). An agar plug of 6 mm diameter was taken from ISP2 agar plate of *Streptomyces* and transferred onto CAS medium, and incubated for 1 week at 30℃. The positive siderophore activity was observed by the presence of orange color halo of colony.

Ammonia production

20 µl *Streptomyces* culture was transferred to 10 ml peptone solution, and incubated in rotary shaker at 30℃ with 120 rpm for 15 days. A culture mixture with 0.5ml Nesseler's reagent was applied. A positive test for the development of ammonia indicated the formation of brown to yellow colors. The absorbance at 530 nm was measured using spectrophotometer, and compared with the standard curve of ammonium sulphate $(NH_4)_2SO_4$ which expressed in mg/ml (Cappuccino and Sherman, 1992).

Hydrolytic enzyme production

The chitinase production was evaluated on chitin agar medium using the substrate colloidal chitin (Hoster *et al*., 2005). The cellulase activity was determined on mineral salt agar containing carboxy methyl cellulose (CMC) as carbon source. The activities of amylase and protease were determined using starch agar and skim milk agar media (Radhakrishnan *et al*., 2007).

Studies on plant growth promotion

Plant growth promotion of *Streptomyces* strains were evaluated using paper towel method (ISTA, 1993). The seeds of *Capsicum annum* were surface-sterilized for 3 min with 1% sodium hypochlorite and rinsed with steriled water. Seeds were separately placed in a suspension (~10 conidia ml^{-1}) of *Streptomyces* strain for 30 min in aseptic condition (Hoyos-Carvajal *et al.*, 2009). Steriled water was used in control seeds. The tests were performed twice with ten seeds per treatment. The shoot and root lengths were measured after two weeks incubation at 28±2 ℃. Percent germination rate was calculated.

Production and identification of volatile compounds from potential Streptomyces sp.

Streptomyces strain UC1A-3 was grown on ISP2 agar medium at 28℃ for 7 days. After 7 days incubation, the *Streptomyces* strain UC1A-3 was transferred to 250 ml flask containing 40 ml ISP2 broth and incubated at 28℃ with agitation speed of 160 rpm for 48 hrs as seed culture.

Antimicrobial compound production was performed in 500 ml fermentation medium which consisted of 47 g soluble starch, 22 g soybean meal, 2.7 g $(NH_4)_2\ SO_4$, 2.7 g NaCl, 2.7 g $CaCO_3$ and 3 g yeast extract, then dissolved in 1 L distilled water and adjusted pH to 6.8 - 7.2 and sterilized. The medium was transferred with 5% (v/v) seed culture after sterilization and incubated at 28℃ in rotator shaker with agitation speed of 160 rpm for 96 hrs. Culture was centrifuged after fermentation and extracted the supernatant twice with ethyl acetate. A proportion of 1:1 (v/v), the solvent was added to filtrate

thorough shaker for 20 min, and incubated overnight. The antibiotic metabolites containing ethyl acetate phase was divided by a separating funnel from the aqueous phase. Ethyl acetate layer was concentrated using rotary evaporator**.** The residue was dissolved using ethyl acetate to brown crude extract. The crude extract was identified the bioactive compounds using GC-MS technique. Agilent technologies (6890–5973 N) with capillary column TG-5 MS phenyl methyl siloxane (30m × 250µm × 0.25µm) system. A split mode mass detector and a 1.0 ml/min flow rate of helium gas was used as a carrier. An injector was operated at 230 ℃ with an initial set-up temperature at 60 ℃ for 2 min, and a ramp of 10/min at 280 ℃ for 8 min.

Characterization and taxonomy of potential Streptomyces strain

The potential *Streptomyces* strain UC1A-3 was characterized on their phenotypic, microscopic and molecular characteristics. Cultural characteristics were studied by growing the *Streptomyces* strain UC1A-3 on ISP1 – ISP7 agar plates. The results were noted after incubation at 28 ℃ for 7-14 days. The micromorphology of the strainUC1A-3 was examined using bright field microscope. ISP2 agar medium was used for utilization of sugar (glucose, sucrose, rhamnose, xylose, raffinose, inositol, cellulose, and mannitol) and amino acid (asparagines, glutamine and tyrosine) (Shirling and Gottileb, 1966).

The total genomic DNA extraction from the strain UC1A-3 was done using solute ready genomic DNA kit (Hi-media). The 16S rRNA gene of UC1A-3 was amplified by using the primer pairs: 27F 5 ́AGAGTTTGATCMTGGCTCAG3 ´ (forward) and 1492R 5 ̀TACGGYTACCTTGTTACGACTT3 ´(reverse) and sequencing was carried out at Eurofins Genomics India Pvt. Ltd., Bangalore. The identification of phylogenetic neighbors and calculation of pair wise 16SrRNA gene sequence similarities were achieved using the MEGA version 6 and BLAST analysis (http://blast.ncbi.nlm.nihgov/Blast.cgi). The 16SrRNA sequence was deposited to GenBank to receive the accession number.

Statistical analysis

The collected data were analyzed using the general linear Agres and Agdata model software. The means were compared using LSD at probability level <0.05 in order to evaluate the effects of the *Streptomyces* sp. UC1A3 to promote plant growth.

Results

Isolation and characterization of actinobacteria

Fourteen morphologically dissimilar Actinobacterial strains were selected from numerous colonies observed on starch casein agar media. The cultural and morphological features of all the actinobacterial strains were given in Table 1. On ISP2 agar medium, ten actinobacterial strains were produced leathery growth whereas the remaining four strains produced powdery growth. All strains showed aerial and substrate mycelia under bright field microscope. Actinobacteria were isolated in SCA showing the most predominantly used media and may increase the possibility to isolate the different *Streptomyces* species. The distinct nutritional content in isolation media mainly in carbon sources may encourage the growth of different *Streptomyces* species, thus increasing the diversity of *Streptomyces* species.

In-vitro screening for antagonistic potential

Among the fourteen strains tested for antagonistic potential, three isolates (21.42%) showed actively against at least one of the phytopathogens (Table 2). Notably, the strain UC1A-3 showed antagonistic activity against all the six tested plant pathogens. It showed maximum zone of inhibition against bacterial plant pathogens via., *Ralstonia solanacearum* (28.2±0.3 mm) and *Xandhomonas oryzae* (21.5±0.8 mm); fungal plant pathogens *viz.*, *Fusarium oxysporum* (40.6±1.2 %), *Alternaria* sp. (58.4±0.9 %), *Macrophomina* sp. (46.1±1.1 %) and *Magnaporthe oryzae* (72.0±0.9 %).

In-vitro plant growth promotion and enzymatic attributes of the actinobacteria

Among fourteen actinobacteria strains, eleven stains were able to produce IAA (Table 3). Strain UP1A3 (61.8±3.2 µg/ml) produced maximum amount of IAA followed by UP1A-7 (56.2±4.4 µg/ml), UP2A-8 (44.3±1.1 µg/ml) and UP2A-9 (42.5±2.1 µg/ml) (fig. 1). In addition, strain UP2A9 was able to soluble maximum amount of phosphate (104±9.9 µg/ml) followed by UP1A3 (103.8±6.7 µg/ml) and UP2A8 (101.2±6.1 µg/ml) (Table 3). Similarly, UP1A-3 (49.8±3.2 µg/ml) produced maximum amount of ammonia followed by UP1A-1 (46.7±1.5 µg/ml), UP1A-6 (40.2±1.6 µg/ml) and UP2A-8 (46.5±1.5 µg/ml) (Table 3 and Figure 1). Moreover, the strains UP1A-1, UP1A3, UP1A-4 and UP2A-12 were produced all other plant growth promotion (PGP) and enzyme properties via siderophore, amylase, protease and cellulase (Figure 1).

Table 1. Cultural Characteristic of Actinobacterial strains isolated from agricultural rhizosphire soil

S. No	Strain no*	Growth	Colony Consistency	Aerial Mass Color	Reverse Side Pigment	Soluble pigment	AM	SM	Suspected genera
1	UC1A-1	Good	Leathery	White	-	-	+	+	*Streptomyces* species
2	UC1A-2	Good	Leathery	Pale Pink White	-	-	+	+	*Streptomyces* species
3	UC1A-3	Good	Powdery	Pale White	-	-	+	+	*Streptomyces* species
4	UC1A-4	Good	Leathery	Colorless	-	-	+	+	*Streptomyces* species
5	UC1A-5	Moderate	Rough, Leathery	Brown	Brown	-	+	+	*Streptomyces* species
6	UC1A-6	Good	Leathery	Colorless	Yellow	Yellow	+	+	*Streptomyces* species
7	UC1A-7	Moderate	Leathery	White	-	-	+	+	*Streptomyces* species
8	UC2A-8	Moderate	Leathery	Colorless	-	-	+	+	*Streptomyces* species
9	UC2A-9	Good	Leathery	Pink White	Pink	Pink	+	+	*Streptomyces* species
10	UC2A-10	Good	Powdery	Ash White	-	-	+	+	*Streptomyces* species
11	UC2A-11	Good	Powdery	Ash White	Yellow	Yellow	+	+	*Streptomyces* species
12	UC2A-12	Good	Powdery	Ash	-	-	+	+	*Streptomyces* species
13	UC2A-13	Good	Smooth, Leathery	Dirty White	-	-	+	+	*Streptomyces* species
14	UC1A-14	Good	Rough, Leathery	Dirty White	-	-	+	+	*Streptomyces* species

*UC1A-1= Udhagamandalam Chilli1 Actinobacteria1

Table 2. *In-vitro* PGP and antibiotic sensitivity of actinobacterial strains isolated from rhizosphere soil

S. No	Strain	Plant Growth Promoting Traits (PGPR)						
		IAA	**PO4 Solubilization**	**Siderophore**	**Ammonia**	**Amylase**	**Protease**	**Cellulase**
1	UP1A-1	39.3 ±1.8	54.7 ±5.6	1	46.7 ±1.5	1	2	1
2	UP1A-2	6.8 ±1.5	3.8 ±0.9	0	0.0	0	1	3
3	UP1A-3	61.8 ±3.2	103.8 ±6.7	1	49.8 ±3.2	2	2	2
4	UP1A-4	35.8 ±0.7	39.9 ±2.6	1	31.2 ±1.6	1	1	1
5	UP1A-5	32.5 ±1.2	0.0	0	10.5 ±3.0	0	1	0
6	UP1A-6	14.0 ±1.0	0.0	0	40.2 ±1.6	1	0	0
7	UP1A-7	56.2 ±4.4	0.0	1	0.0	0	0	1
8	UP2A-8	44.3 ±1.1	101.2 ±6.1	0	46.5 ±1.5	2	1	1
9	UP2A-9	42.5 ±2.1	104 ±9.9	0	16.7 ±1.7	1	2	1
10	UP2A-10	3.8 ±1.5	0.0	0	14.8 ±0.6	0	1	0
11	UP2A-11	0	10.6 ±1.2	0	0.0	0	0	1
12	UP2A-12	0	0.0	1	27.7 ±1.2	1	2	2
13	UP2A-13	0	0.0	0	24.2 ±2.0	0	3	2
14	UC1A-14	8.0 ±1.8	0.0	1	0.0	0	1	0

The rating scales for siderophore, chitinase, cellulase, lipase and protease were as follows: 0 = no halo zone, 1 = halo zone of 1–10 mm, 2 = halo zone of 11–20 mm, 3 = halo zone of 21–30 mm, 4 = halo zone of 31–40 mm

Table 3. *In vitro* screening of actinobacterial strains for antimicrobial activity

S. No	Strains	Bacterial plant pathogens (mm)		Fungal plant pathogens (%)			
		Ralstonia solanacearum	***Xandhomonas oryzae***	***Fusarium oxysporum***	***Alternaria* sp.**	***Macrophomina* sp.**	***Magnaporthe oryzae***
1	UC1A-3	28.2±0.3	21.5±0.8	40.6±1.2	58.4±0.9	46.1±1.1	72.0±0.9
2	UC2A-8	-	-	-	42.2±0.6	-	-
3	UC2A-9	-	-	-	-	-	55.5±0.8

Figure 1. *In-vitro* plant growth promotion and enzyme activities of potential *Streptomyces* sp. UT3A-39: Production of a. IAA, b. Siderophore, c. Ammonia, Amylase, Protease, Cellulose

In-vitro plant growth promotion by paper towel method

Totally three potential *Streptomyces* strains were tested *in-vitro* PGP and antagonistic activity for chilli plant growth promotion using paper towel method. Out of three strains, UC1A3 showed highest germination percentage in chilli (89%), and followed by UC2A8 (70.3%) and UC2A9 (50.3%) (Table 4). UC1A3 showed the highest shoot length (45.6 cm), and followed by UC2A8 (44.9%) UC2A9 (39.2%) when compared to control treatment (32.7%). Moreover, UC2A8 showed more root length than other *Streptomyces* treated and control chilli seeds (Table 4).

Table 4. *In-vitro* PGP activity of *Streptomyces* strains using paper towel method

Strains	Germination %	Shoot length (mm)	Root length (mm)
UC1A3	89[a]	45.6[a]	9.3[b]
UC2A8	70.3[b]	44.9[a]	10.1[a]
UC2A9	50.3[c]	39.2[b]	5.5[c]
Control	49.6[c]	32.7[c]	5.1[c]

Identification of volatile compounds

The GC-MS technique of ethyl acetate extract of *Streptomyces* sp. UC1A3 confirmed the presence of compounds with different retention times as illustrated in Figure 2. The identified compounds and their retention time, molecular mass, peak area (%), molecular formula, and activities are given in Table 5. Twenty nine compounds were elucidated and mostly found the derivatives of aromatic compounds. Phthalic acid ($C_8H_6O_4$), Pentadecanoic acid ($C_{15}H_{30}O_2$), i-Propyl 12-methyltetradecanoate ($C_{18}H_{36}O_2$), l-(+)-Ascorbic acid 2,6-dihexadecanoate ($C_{38}H_{68}O_8$), 1-Nonadecene ($C_{19}H_{38}$), 1-Heptacosanol ($C_{27}H_{56}O$) showed antagonistic properties with highest peak number (Figure 3). The most prevailing major compounds were l-(+)-Ascorbic acid 2,6-dihexadecanoate (peak area: 14.14%), i-Propyl 12-methyltetradecanoate (peak area: 13.62%) and 1-Heptacosanol (peak area: 7.28%).

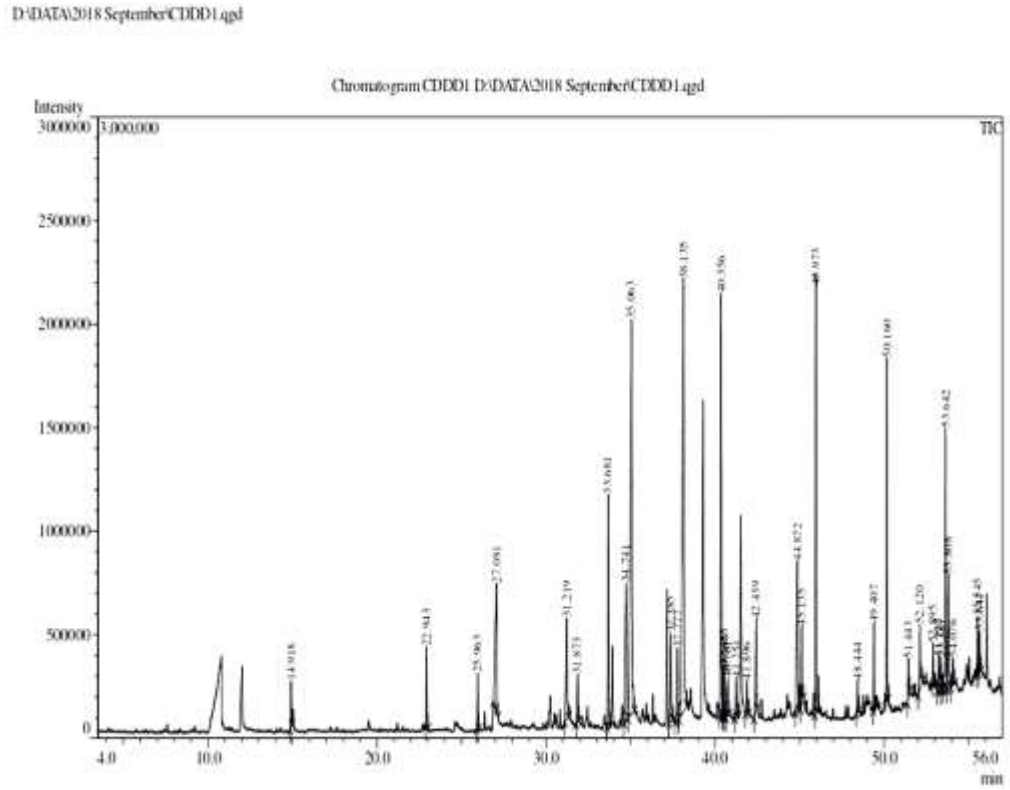

Figure 2. GC-MS chromatogram of ethyl acetate extracts of *Streptomyces* sp. UC1A3

Table 5. Compounds identified from the crude extract of *Streptomyces* sp. UC1A-3 through GC-MS

Compound name	Retention Time	MM	Area %	MF	Activity	References
1,2-Benzenedicarboxylic acid	14.918	166	0.81	C8H6O4	Anticancer activity	Krishnan et al., 2014
Phenol, 2,4-bis(1,1-dimethylethyl)-	22.943	206	1.4	C14H22O	Anti- quorum sensing and Anti-biofilm	Padmavathi et al., 2014
Diethyl Phthalate	25.963	222	0.92	C12H14O4	Antimicrobial activity	Premjanu and Jaynthy., 2014
Phthalic acid, monoethyl ester	27.081	194	5.03	C10H10O4	Biodegradation	Singh et al., 2017
9-Octadecenoic acid	31.219	282	2.6	C18H34O2	Anti-inflammatory, antiandrogenic cancer preventive, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge	Krishnamoorthy et al., 2014
Octahydro-4a(2h)-naphthaleno	31.873	154	1.24	C10H18O	No activity	-
1-Nonadecene	33.681	266	4.42	C19H38	Antituberculosis, anticancer, antioxidant, antimicrobial	Rukachaisirikul et al., 2004; Lee et al., 2007
Pentadecanoic acid	34.741	242	4.66	C15H30O2	Lubricants, Adhesive agents, Antioxidant.	Sunita Arora et al., 2017; Elezabeth and Arumugam, 2014
i-Propyl 12-methyltetradecanoate	35.063	284	13.62	C18H36O2	No activity	-
cis-9-Hexadecenoic acid	37.385	254	2	C16H30O2	anti-inflammatory activity	Astudillo et al., 2018
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	37.777	210	1.91	C11H18N2O2	Cytotoxicity, genotoxicity activity, hemolytic activity	Sanjenbam and Kannabiran, 2016
l-(+)-Ascorbic acid 2,6-dihexadecanoate	38.135	652	14.14	C38H68O8	Antioxidant, antiinflammatory and anti-nociceptive properties	Okwu & Ighodaro 2010
cis-10-Heptadecenoic acid	40.468	268	0.94	C17H32O2	antitumor activity	Fukuzawa et al., 2008

Table 5. (Con.)

Compound name	Retention Time	MM	Area %	MF	Activity	References
Heneicosane	40.568	296	0.88	C21H44	No activity	-
1,13-Tetradecadien-3-one	40.761	208	1.02	C14H240	No activity	-
Oleic Acid	41.896	282	0.71	C18H34O2	Antibacterial	Awa et al., 2012
cis-4a-Methyl-1,2,3,4,4a,9a-hexahydrocarba	42.439	187	2.14	C13H17N	No activity	-
3,6-Diisobutyl-2,5-piperazinedion	44.872	226	3.93	C12H22N2O2	No activity	-
Octadecanoic acid	45.135	284	1.38	C18H36O2	Antimicrobial activity	Rahuman et al., 2000
1-Heptacosanol	45.973	396	7.28	C27H56O	Antioxidant and antimicrobial	Al-Abd et al., 2015
Ergotaman-3',6',18-trione, 9,10-dihydro-12'-	48.444	583	0.62	C33H37N5O5	Antimicrobial activity	Zote et al., 2018
3-benzylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione	49.407	244	1.37	C14H16N2O2	Antibiofilm activity	Singh et al., 2019
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	51.443	330	0.63	C19H38O4	Antimicrobial	Tyagi et al., 2017
3-benzyl-6-isobutyl-2,5-dioxo-piperazine	52.895	260	0.73	C15H20N2O2	Antimicrobial	Kumari et al., 2019
Octadecanoic acid, 2,3-dihydroxypropyl este	53.227	358	0.81	C21H42O4	Anticancer, antimicrobial	Arora et al., 2017
Heneicosyl trifluoroacetate	53.642	408	3.91	C23H43F3O2	No activity	-
Tetrapentacontane	54.076	758	0.61	C54H110	Antioxident and antimicrobial	Swamy et a., 2017
2,6-dodecadien-1-ol, 3,7,11-trimethyl-, (e,e)	55.545	224	1	C15H28O	No activity	-
Octahydro-2H-pyrido(1,2-a)pyrimidin-2-one	55.641	154	0.81	C8H14N2O	No activity	-

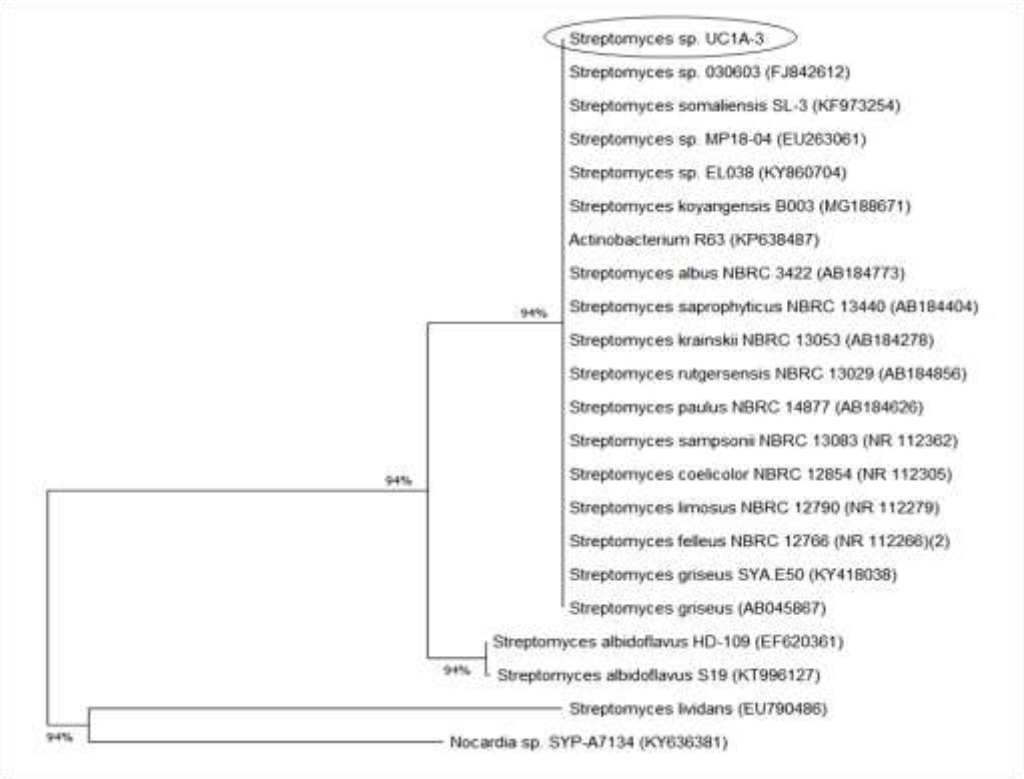

Figure 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences shows the strain *Streptomyces* sp. UC1A3 to be a sister taxa of various species of *Streptomyces* within the family Streptomycetaceae. *Nocardia* sp. SYP-A7134 was used as the out-group

Characterization and taxonomy of strain UC1A3

The studied PGP and biocontrol agent strain UC1A3 formed an extensively branched substrate mycelia, aerial hyphae which carried smooth-surfaced spores in spirals shaped spore chains. The strain UC1A3 proved to produce good growth in tryptone yeast agar, yeast-malt agar, oat meal agar, inorganic salts - starch agar and tyrosine agar medium and moderate growth in glycerol asparagine agar and peptone yeast iron agar. Morevere, sucrose, inositol, mannitol, rhamnose, asparagines and glutamine were influenced the growth of UC1A3 (Table 6). The UC1A3 strain has been tentatively described as a genus of *Streptomyces* based on the morphological and physiological characteristics. The 16S rRNA gene sequence of strain UC1A3 was compared to the similar sequences of *Streptomyces* already deposited in NCBI GenBank database, using BLASTn (NCBI website). The strain displayed 1447 nucleotide length and 99.59% similarity with *Streptomyces* sp. (mention the closely related

species of *Streptomyces* instead of putting as sp.). The 16S rRNA sequence of the strain UC1A3 was deposited in NCBI-GenBank under accession number MN715830. UPGMA clustering grouped the *Streptomyces* strain UC1A3 with other known *Streptomyces* species collected from NCBI (Figure 3).

Table 6. Cultural and other characteristic of *Streptomyces* sp. UC1A3

Characteristics	***Streptomyces* sp. UC1A3**
Growth	Good
Consistency	Powdery
Aerial mycelium color	Grey
Soluble pigment	Nil
Reverse side pigment	Nil
Micro morphology	
Aerial mycelium	Present
substrate mycelium	Present
spore chain morphology	Spirals
ISP Medium	
Tryptone yeast extract agar	Good
Yeast extract malt extract agar	Good
Oat Meal agar	Good
Inorganic salts - starch agar	Good
Glycerol asparagine agar	Moderate
Peptone yeast extract iron agar	Moderate
Tyrosine agar	Good
Factors & Variables	
Sugars	
Glucose	Moderate
Sucrose	Good
Xylose	Poor
Inositol	Good
Mannitol	Good
Rhamnose	Good
Raffinose	Poor
Cellulose	-
Amino acid	
Asparagine	Good
Glutamine	Good
Tyrosine	Poor

Discussion

The results stated that morphological features of all the 14 actinobacterial strains belong to *Streptomyces* (Williams, 1989). The above results suggested that UC1A-3 is considered as a potent agent to control and/or reduce the diseases of plants caused by fungal and bacterial pathogens. Many researchers are studied on the potential of *Streptomyces* species to reduce the population of *R. solanacearum* caused bacterial wilt (Tan *et al*., 2006), bacterial leaf blight of rice caused by *X. oryzae* (Hastuti *et al*., 2012), wilt caused *F. oxysporum* (Zhao *et al*., 2012), tobacco brown spot disease caused by *Alternaria* sp. (Gao *et al.,* 2014), antagonistic activity against *Macrophomina* sp. (Shrivastava *et al*., 2017) and rice blast caused by *Magnaporthe oryzae* (Law *et al*., 2017). It is shown that nearly 80% of isolates from the rhizosphere soil can be synthesized IAA as secondary metabolites to promote root elongation and plant growth (Patten and Glick, 2002). The plant hormones production promotes the growth of plants by microorganisms to increase the plant metabolite activities. Our research finding, the differences in IAA production among *Streptomyces* strains are influenced by genetic and metabolic background to convert L-tryptophan to IAA. IAA production was found by the isolated strains in this study is comparable reported by Verma *et al*. (2011) and Khamna *et al*. (2009). Our results are also in agreed with Hamdali *et al*. (2008) who reported that *S. cavourensis* and *S. griseus* were capable to phosphate solubilization at 83.3 and 58.9 mg/100 ml, respectively. Similarly, Shrivastava *et al*. (2017) reported that halotolerant *S. aureofaciens* produced IAA, phosphate solubilisation, siderophore, ammonia and hydrolytic enzymes such as amylase, chitinase and urease. Wahyudi *et al*. (2019) reported that IAA-producing actinobacteria strains were enhanced better plant shoot and root length as compared to un-inoculated plants and to promote biocontrol activity of chilli using *Streptomyces* that is limited when it compared with *inplanta*. Also, there are several other studies proved that *Streptomyces* strains were significantly enhanced both root and shoot lengths (Sreevidya *et al*., 2015; Gholami *et al*., 2009). Although, due to its direct and indirect mechanisms, *Streptomyces* has become widely applied to promote the growth of plants and to control phytopathogens (Vurukonda *et al*., 2018).

GC-MS is one of the powerful analytical techniques for the analysis of microbial secondary metabolites (Tan *et al*., 2015). It also performs an important role in the discovery of natural products such as bioactive compounds derived from *Streptomyces* (Ara *et al*., 2014; Jog *et al*., 2014). Sanjenbam and Kannabiran (2016) reported the detection of an antifungal bioactive compound, isolated from *Streptomyces* sp. Chen *et al*. (2018) found

18 chemical compounds with different retention times and relative abundances from *Streptomyces* using GC-MS. Moreover, 1,2-benzenedicarboxylic acid isolated from *Streptomyces* sp. was actively cytotoxicity against MCF- 7 and HepG2 cancer cell lines (Krishnan *et al*., 2014). Likewise, phenol, 2,4-bis(1,1-dimethylethyl) was found to be an efficient anti-pathogenic agent isolated from *Streptomyces* (Padmavathi *et al*., 2014). Previous reports by Chen *et al*. (2018), Tan *et al*. (2015) and Ser *et al*. (2015) showed the importance of GC-MS characterization for common effect of bioactive compounds identification. Similarly, 1-Nonadecene was found to be anti-tubercular, anticancer, antioxidant, antimicrobial potential against various pathogens (Rukachaisirikul *et al*., 2004; Lee *et al*., 2007). Thus, the present study revealed that twenty nine compounds were identified through GC-MS could be the key contributing factors to the bioactivity of UC1A3. Thus, it is in agreement with the previous reports proved that *Streptomyces* are known to be leading species among the actinobacterial phylum with the production of antimicrobial metabolites (Khamna *et al*., 2009; Kaur *et al*., 2013; Rashad *et al*., 2015). The 16S rRNA gene sequence analysis has confirmed the classification of the strain to the *Streptomyces*. The method has been used as a common and basic approach to identify the microorganisms and studying their diversity from natural diverse ecosystems (Solanki *et al*., 2014). Hence, *Streptomyces* sp. UC1A3 is a promising microbial candidate for agricultural applications since it showed good anti-pathogens and promoting plant growth.

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