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Isolation and Characterization of *Streptomyces chartreusis* DTPI13, a Non-*Frankia* Endophytic Actinobacterium from *Casuarina cunninghamiana* Root Nodules, with Plant Growth-Promoting Potential

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Abstract

Actinorhizal plants comprise eight genera distributed across three orders: Fagales, Rosales and Cucurbitales. These plants form root nodules that harbour endophytic bacteria, particularly *Frankia*, which establishes a well-known nitrogen-fixing symbiosis. This association enables host plants to adapt to and colonize challenging environments. However, non-*Frankia* bacteria have also been reported in actinorhizal root nodules, although their contributions to plant–microbe interactions remain poorly understood. The present study aimed to isolate and characterize non-*Frankia* endophytic actinobacteria from surface-sterilized root nodules of *Casuarina cunninghamiana* and evaluate their plant growth-promoting potential. Following isolation and validation of their endophytic nature, selected isolates were subjected to morphological, molecular and physiological characterization. Isolate DTPI13 was identified as *Streptomyces chartreusis* based on morphological characteristics and 16S rRNA gene amplification and sequencing. Its plant growth-promoting traits were investigated by assessing indole-3-acetic acid (IAA) and ammonia production, siderophore and hydrogen cyanide production, organic acid production, phosphate solubilization, ACC deaminase activity and hydrolytic enzyme activities. The isolate was also characterized for its cultural and physiological properties. The investigation revealed the presence of a non-*Frankia* endophytic *Streptomyces* associated with *C. cunninghamiana* root nodules and provided a preliminary assessment of its plant growth-promoting attributes. These findings expand the understanding of the actinobacterial community associated with actinorhizal root nodules and suggest that *S. chartreusis* may have potential as a plant growth-promoting helper bacterium. Further studies are required to establish whether this isolate directly enhances *Frankia*-mediated symbiosis and contributes to host plant growth and adaptation under environmental stress under saline coastal conditions through complementary microbial interactions.

1. INTRODUCTION

Actinorhizal plants, which include over 200 distinct plant species in 25 genera and eight families, are distinguished from other divergent dicotyledonous plants by their tendency to establish a symbiotic relationship with actinobacteria from the genus *Frankia* (Benson and Silvester, 1993). Actinorhizal plant family *Casurinaceae* contains three genera *Casuarina*, *Allocasuarina*, and Gymnostoma. *Casuarina* is a fast growing, drought hardy and able to develop a nitrogen fixing symbiosis with the Actinobacteria. Actinobacteria represent approximately 20–30% of the rhizospheric microbial community (Bouizgarne and Ben Aouamar, 2014). They are Gram-positive and show a wide morphological spectrum ranging from unicellular organisms to branching filaments that form a mycelium. A unique feature is their high guanine plus cytosine content (>50%) in their genome (Trujillo *et al*., 2015). In the rhizosphere of plants, actinobacteria are widely distributed and produce a variety of agro-active substances. Due to its potent antimicrobial properties and dominant saprophytic nature in the soil, this particular genus has

received a lot of interest recently as a plant growth stimulator. Plant growth-promoting rhizobacteria (PGPR) are naturally occurring, free-living rhizosphere-colonizing microorganisms that enhance soil fertility, promote plant development, supress pathogens, and mitigate biotic and abiotic stresses (Anwar *et al*., 2016). In relation to the extensive distribution of actinobacterial genera among PGPB and the high population of these bacteria in soil (10^{-7}-10^{-8} per g^{-l}) in comparison to other bacteria (10^{-8}-10^{-9}per g–l). Actinobacterial species known as plant growth promoting actinobacteria (PGPA) have an effective symbiotic association with plants and optimise the plants by generating plant growth hormones like indole acetic acid (IAA), cytokinin's, and gibberellins, solubilizing inorganic phosphate, asymbiotic nitrogen fixation, antagonistic effect against phytopathogenic microorganisms by generating siderophores, antibiotics, and fungicidal compounds, and more (Hamedi *et al*., 2015 and Anwar *et al*., 2016). *Frankia* is well known N_2 fixing symbionts in the root nodules of actinorhizal plants. It has been reported in several Actinorhizal plants in *Casuarina cunninghamiana, Casuarina equisetifolia, Casuarina glauca* (Dwight baker *et.al,* 1980, Mastronunzio et.al, 2009). In our previous study, we have reported *Frankia* isolated from *Casuarina cunninghamiana* and analysed the morphology, molecular characterization and plant growth promoting activity (Narayanasamy *et al*., 2020; 2021). There are several non-frankial actinobacterial genera which helps in effective symbiotic association of *Frankia* and actinorhizal plant *Casuarina* sp. which includes *Streptomyces, Nocardia Thermomonospora, Spirillospora, Micromonospora and Salinospora (*aria Valdes *et.al,* 2004). These actinobacteria may also serve as "helping bacteria" that facilitate *Frankia's* infection and nodulation of its host plant (Gtari and Tisa, 2014). We are therefore curious to learn more about the non- Frankial population in the *Casuarina* plant's root nodules. In the root nodules of the actinorhizal plant *Casuarina*, we intend to explore the endophytic actinobacteria as helper symbiotic actinobacteria with *Frankia*.

2. MATERIALS & METHODS

2.1 Root nodule sampling site

The study area is located at the Pichavaram mangrove forest is located in Cuddalore district, Tamil Nadu, India, between Latitude: 11° 26' 16.476" N and Longitude: 79° 43' 9.84" E. Furthermore, it is 3.0 meters above sea level. *Casuarina cunninghamiana* fresh nodules associated to the root system were sampled with small sections of roots at a depth of 10-30 cm from the topsoil surface. The healthy light brown nodules were identified and transported to the laboratory under aseptic conditions and stored at 4° C. Within 24 hours of collection, the samples were processed for the isolation of actinobacteria.

2.2 Isolation of endophytic actinobacteria

Harvested nodules were cleaned thoroughly with sterile tap water to remove soil, dust particles and sterilized superficially by shaking in 20 % (v/v) H_2O_2 for 8 min and then aseptically rinsed several times with sterile water. Only nodules resulting in a sterile final washing liquid were further

considered for actinobacterial isolation in SCA medium. Surface sterilized root samples were crushed and serially diluted. The dilutions were spread onto SCA supplemented with antibiotics (Amphotericin & Cycloheximide). Endophytic actinobacteria were observed after incubation at 28 °C for 7 days. Isolates which exhibited good growth within 4 days were considered as fast growers, the isolates those revealed good growth between 4th to 7th days of incubation were considered as moderate growers and the isolates those raised after 7 days was considered as slow grower (Rijavec and Lapanje, 2016).

2.3 Cultural & morphological characterization

The isolates were identified according to morphological criteria, including form, shape, size, elevation, texture, opacity and margin of colony, colour of substrate and aerial hyphae (Goodfellow and Cross, 1984). Cultural and morphological characteristics of isolate DTPI13 were determined after 7 days at 28°C on standard ISP-1 (tryptone yeast extract broth agar), ISP-2 (yeast extract-malt extract agar), ISP-3 (oatmeal agar), ISP-4 (inorganic salts starch agar), ISP-5 (glycerol-asparagine agar), ISP-6 (peptone-yeast extract-iron agar) and ISP-7 (tyrosine agar) media (Shirling and Gottlieb 1966). The production of melanin is an important characteristic for the identification and classification of actinobacteria, especially *Streptomyces*. Melanin is a dark pigment produced by the microorganisms on tyrosine containing a medium. The tryptone yeast extract broth (ISP-1), yeast extract iron agar (ISP-6), tyrosine agar (ISP-7) was used for testing melanin production by the actinobacteria isolates. Seven-day old culture of actinobacteria isolates was inoculated on each media and incubated at 27 °C for 4 d and the uninoculated media serves as a control. The colour change was observed after 2 d and 4 d. greenish brown and brown to black diffusible pigments were considered as positive for melanin production (Li et al., 2016).

2.4 Morphological characterization by microscopic coverslip culture method

The arrangement of spores and sporulating properties of the selected isolates were examined microscopically by using cover slip culture method by inserting sterile cover slip at an angle of 45 ° in the starch casein agar medium. A loop full of isolate was taken separately from 7 d old culture, inoculated at the insertion of coverslip and incubated at 27 °C for 7 d. Then, the coverslip was removed by using sterile forceps and placed upward on a clean glass slide. Finally, the cover slip was observed for the morphology of isolates under the light microscope at 400 magnifications (Williams & Cross, 1971).

2.5 Characterization of plant growth promoting traits involved in Microbe–Plant Interactions

2.5.1 Ammonia production, according to Islam *et al* (2009) guidelines, actinobacteria isolates were evaluated for their ability to produce ammonia. Actinobacteria cultures were newly produced and inoculated to 10 ml of peptone water. The test tubes that had been seeded were kept at 25°C for 1 ml of Nessler's reagent was added to the sample after 7 days. Every test tube was examined for any colour changes. A change in the colour of the media to yellow or brown specifies a successful outcome for ammonia

synthesis. The assessment was completed triplicate.

2.5.2 Phosphate solubilization, Using the approach described by Islam *et al*. (2009), the capacity of actinobacteria isolates to solubilize phosphate was assessed. On Pikovskaya's agar plates with 2% tri-calcium phosphate, 10 ml of freshly made culture was spot loaded. After seven days of incubation at 37°C, inoculated plates were checked to determine if a clear zone had formed around at the actinobacteria colonies.

2.5.3 Hydrogen cyanide production, Separate streaks of bacterial cultures were placed on Luria Bertani (LB) agar that had had 0.4 percent (w/v) glycine added. The lid of the Petri dish was covered with a Whatman no. 1 filter paper that had been dipped in a solution of 0.5 percent (w/v) picric acid and 2 percent (w/v) sodium carbonate. Plates were then securely parafilm sealed and housed for seven days. When seen with the eyes, the filter paper's transition from yellow to deep orange denotes a HCN produced by the actinobacteria (Bakker and Schippers, 1987).

2.5.4 IAA production, 20 ml of LB broth with 5 mmol L-tryptophan added to it was combined with 20 ml of freshly generated bacterial cultures, and the combination was then incubated at 25 °C for 7 days. The supernatant was obtained after centrifuging 1 ml of the bacterial culture at 5000 g for 15 min. Then, 2 ml of the supernatant and a few drops of orthophosphoric acid were added along with 4 ml of the Salkowsky reagent (50 ml of 35 percent perchloric acid in 1 ml of 0.5 M $FeCl_3$). The creation of a pink tint showed that the contents of the tubes had created IAA after 20 minutes of incubation at room temperature and in the dark. A UV spectrophotometer was used to determine the pink colour's absorption at 530 nm (Ndeddy Aka and Babalola's, 2016)

2.5.5 Siderophore production, A modified method published by Schwyn and Neilands (1987) was used to assess the siderophore production of bacterial isolates using the indicator dye chrome azurol S. In a nutshell, 60.5 mg of CAS was dissolved in 50 ml of distilled water, and 10 ml of iron (III) solution was then added. In 40 ml of distilled water, we dissolved 72.9 mg of hexadecyltrimethylammonium bromide. The mixture was then added laterally while being continually agitated with a magnetic stirrer. After that, the combination underwent a 15-minute autoclave at 121 °C. The finished combination (100 ml) was added while stirring to 900 ml of sterile LB broth. Freshly made bacterial cultures were spot-inoculated onto the Petri plates and kept for seven days at 25°C. A yellowish-orange ring surrounding the bacterial colonies was seen as a sign that siderophore synthesis had succeeded.

2.5.6 ACC deaminase activity, first, all the bacteria were cultured for 48 hours at room temperature in 5 ml of tryptone-soy broth. To get bacterial cells, centrifuge them at 5000 g for five minutes, rinse them twice in sterile 0.1 M Tris-HCl (pH 7.5), and then resuspend them in 1 ml of 0.1 M Tris-HCl (pH 7.5). Washed bacterial cells were spot-injected on Petri plates using modified Dworkin and Foster salts minimal medium (Dworkin and Foster 1958) with 3 mM ACC as the only nitrogen source. The experiment's negative control consisted of petri plates with just DF minimum salts

medium and no ACC, whereas the experiment's positive control consisted of plate with DF minimal salts medium plus 0.2% (w/v) $(NH_4)_2SO_4$. Petri plates were evaluated based on how much bacterial growth transpired when ACC was the only nitrogen source used. Three times the experiment was run (Ali et al., 2014).

2.5.7 Organic acid production, Spot inoculations of bacterial cultures on MM9 agar medium were used to track pH changes using the indicator dye methyl red, which becomes pink below pH 5.0. Isolates that produce organic acid had a pink zone around the colony (Zhao *et al*., 2011)

2.5.8 Chitinolytic activity, in order to prepare chitin colloidal, 5g of powdered chitin was mixed with 75ml of strong hydrochloric acid over the course of an hour. Make up the volume to 1000ml with distilled water that has been filtered. The filtrate residue was washed with distilled water until it reached a pH of 3.5. After making chitin medium and adding 2gm of chitin colloidal, spot injections of actinobacterial cultures were made and kept at 25˚c for seven days. The chitinolytic index was figured out by looking at the clean zone around the colonies (Halimahtussadiyah *et al*, 1823)

2.5.9 Carboxymethyl cellulase activity, CMC at a concentration of 0.2% served as the only carbon source for the formation of carboxymethyl cellulase agar. Spot plating was performed using actinobacterial isolates. All plates underwent a 7-day incubation period at 27° C. The plates were rinsed with 1M NaCl after being inundated with 0.1 percent Congo red for 15 minutes in order to detect the hydrolysis zone (Johnsen & Krause, 2014).

2.6 Endophytic actinobacteria molecular identification

The protocol outlined by was followed during the extraction of genomic DNA. In a 100 ml conical flask filled with SCA broth, the isolate DTPI13 was cultivated and incubated for 48 hours at 28± 2 °C. After being transferred to a 1.5-ml Eppendorf tube, the culture broth was centrifuged at 12,000 rpm for one minute. By draining the supernatant and suspending the bacteria in 567 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, and 1 l of distilled water), the bacteria were kept alive. Then, 780 µl of the entire volume were added, along with 30 µl of 10% sodium dodecyl sulphate, 3 µl of sodium acetate, 100 µl of 5 M NaCl, 80 µl of CTAB NaCl, and they were thoroughly mixed. They were then incubated at 65 °C for 10 minutes using a hot water bath. After adding an equal volume of chloroform and isoamyl alcohol (24:1; 780 µl), the mixture was centrifuged at 12,000 rpm for 10 min. The aqueous DNA layer was extracted using phenol, chloroform, and isoamyl alcohol (25:24:1) after the supernatant was transferred to another Eppendorf tube. After repeating the DNA extraction process twice, the supernatant was collected. For the precipitation of nucleic acid, 400 µl of isopropanol was added to the resulting supernatant in a fresh Eppendorf tube. The genomic DNA was then kept at 20°C after being eluted with 200 L of 75% ethanol (Azadeh & Meon, 2009). Using universal primers 27F (5′- AGAGTTTGATCCTG GCTCAG-3′) and 1492R (5′ ACGGCTACCTTGTT ACGACTT-3′), the 16S rRNA gene was amplified using PCR. Conditions for the PCR reaction were as follows: Primer annealing took place at 54 °C for 1 min, initial DNA was denatured at

95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, extension took place at 72 °C for 1 min, with a final extension lasting 10 min at 72 °C, and cooling took place at 4 °C. The PCR products that had been amplified were identified using 1.8% agarose gel electrophoresis (Mondal & Rai, 2019).

Phylogenetic relationship of isolate DTPI13

The 16S rRNA gene sequence analysis of the endophytic actinobacteria was submitted to the National Centre for Biotechnology Information (NCBI) GenBank, located in the United States. The basic local alignment search tool (BLAST) search was used to retrieve the nucleotide sequences with the highest degree of similarity from the NCBI database. The most similar sequences from a filter-search of the 16S rRNA gene sequence were used for the phylogenetic analysis and MEGA 5.0 software was used to create the dendrogram with a bootstrap value of 1000 replications (Padhi and Tatung, 2013)

3. RESULT & DISCUSSION

3.1 Isolation of endophytic Actinobacteria from *Casuarina* root nodules

The second-largest mangrove forest in the world, Pichavaram yielded *Casuarina* root nodules for isolation of actinobacteria which is located in the Tamil Nadu, India, and district of Cuddalore. The fresh viable root nodules were processed after surface sterilization. Starch casein agar was employed for endophytic actinobacteria isolation. After a week of incubation at 37° C, we isolated an endophytic actinobacteria isolate DTPI13 from surface sterilized root nodules of Casuarina *cunninghamiana*. The final washing liquid collected after surface sterilization process was plated over a SCA plate for the purpose of validating endophytic property, and even after a month of incubation, there was no sign of microbial growth. This finding showed that all epiphytic bacteria in the root nodules were eliminated by the surface sterilization process. The prevalence of actinobacterial colonies in *Casuarina cunninghamiana* suggests that this host species provides a suitable ecological niche that is home to a variety of prolific endophytic microorganisms.

3.2 Cultural & Morphological characteristics of *Streptomyces chartreusis* DTPI13

We isolated an endophytic actinobacteria isolate DTPI13 from the root nodule of *Casuarina cunninghamiana*. The isolate has a dry, powdery texture, a 3-mm-diameter circular form, and white-coloured aerial hyphae and substrate mycelium. Fuzzy characteristic appearance due to aerial hyphae is the primary character of actinobacterial species. Another distinguishing trait of isolate DTPI13 was its peculiar earthy odour of Actinobacteria. According to International Streptomyces Project reports that the isolate DTPI13's cultural traits were detected on ISP-1 to ISP-7 medium and the colour comparison determined from the ISCC NBS colour charts as the results were shown in (Table 1). The colour of the aerial and substrate changed depending on the medium, the isolate DTPI13 is white in SCA, ISP-1, and ISP-5, but can be bluish white or yellowish white in another ISP medium. SCA is the proven medium that supports growth the most, but ISP-2 and ISP-5 are the least

effective and other ISP medium offer good growth. In all ISP media, the colony was round and the elevation ranged from convex to pulvinate. The texture of the isolate is generally dry and powdery, with the exception of the medium ISP-3, which has a moist, slimy texture. ISP-4 (5mm) had the largest colony size, whereas ISP-1 had the smallest colony size (2mm). Except for ISP-3, which is translucent, rest of the medium had the colony's opaque appearance. Only ISP 6 medium featured diffused pigment. ISP-3 and ISP-6 medium were found to have melanoid pigmentation ranging in coloration from dark brown to maroon. The isolate DTPI13 produced substrate mycelia and aerial hyphae with considerable branching that showed a striking resemblance to the genus *Streptomyces* sp. In order to absorb nutrients for the growth of actinobacteria, the substrate mycelium develops into the medium or on its surface. When the substrate mycelium reaches a specific stage of development and begins to grow into the air, it produces aerial hyphae. The cover slip method was used to discriminate between aerial and substrate mycelia. In a specific stage of development, the aerial hyphae of some genera form spore chains, which are the reproductive hyphae that produce spores. Based on spore chains, the Streptomyces genus produces long chains of more than 50 distinct Polyporus spores. A spiral thread of spores was seen under a light microscope (Fig 1). The isolate DTPI13 could have been connected to the genus *Streptomyces* based on its cultural and physical characteristics.

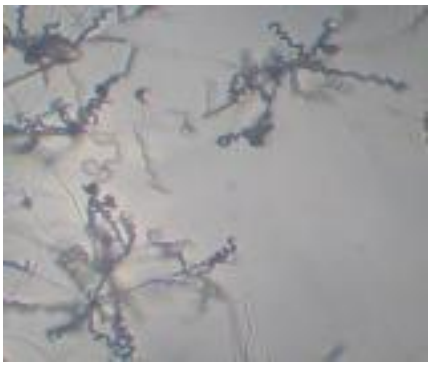


Fig 1: Micromorphology of *Streptomyces chartreusis* DTPI13 on SCA agar

3.3 Plant growth promoting traits of strain DTPI13

Actinobacterial isolate DTPI13 was evaluated for plant growth promoting properties ammonia, indole acetic acid, siderophore, hydrogen cyanide and organic acid production, phosphate solubilization, ACC deaminase activity, chitinolytic activity and carboxymethyl cellulase activity were illustrated in (Table 2). Most living organisms could indeed access atmospheric nitrogen (N_2), but it can be converted to ammonia. The isolate DTPI13 was ammonia-producing positive. Ammonia generation by the isolate in peptone water broth was quantified as 42.3 mg/ml. The enzyme ACC deaminase can break down ACC into ammonia and -Ket butyrate, lowering the quantity of ethylene in the plants. The isolate was placed on DF minimum salt

media, incubated at 28 °C for three days. On the plates, colonies that were growing were identified as ACC deaminase producers. About DTPI13 isolate it is an excellent ACC deaminase producer. Actinorhizal symbioses, among other plant-microbe interactions, are known to benefit from auxin. Spectrophotometrically auxin production in isolate grown in LB broth for seven days at 25°C was detected as 21.1 mg/ml. A significant part of the cell walls of fungi is chitin. Following the measurement of the chitinolytic index, the isolate was cultured on a chitin medium at 25 °C for seven days to exhibit chitinolytic activity. Another documented mechanism of action for endophytic actinobacterial suppression of fungal growth is the production of siderophore (compounds that chelate iron). Colonies were put in CAS-CAA media and examined for the formation of a yellow halo, a sign that siderophores were being produced. Isolate DTPI13 was positive for this test. In order to measure cellulase production, the isolates were cultivated in a minimal medium with CMC serving as the only carbon source. DTPI13 screened positive for this condition. The isolate was able to solubilize phosphate in the Pikovskaya's medium containing tricalcium-phosphate. The change in colour transition of the filter paper after incubation on LB plates with picric acid saturation demonstrated the isolates were HCN producers. DTPI13 isolates' generation of hydrogen cyanide was seen through the yellow picrate filter paper turning red-brown. Incubation on MM9 agar medium with the methyl red as indicator dye, the isolates form pink zone around the colony. The DTPI13 isolate was screened as organic acid producers.

Medium	Growth	Shape	Form	Elevation	Margin	Aerial mycelium	Substrate mycelium	Texture	Size	Opacity	Diffusible pigment	Melanoid pigment
SCA	Excellent	Circular	Circular	Convex	Entire	White	White	Dry powdery	3 mm	Opaque	-	-
ISP1	Good	Circular	Circular	Flat	wavy	White	Pale whitish yellow	Slimy	2 mm	Opaque	-	-
ISP2	Poor	Circular	irregular	Pulvinate	Wavy	Bluish white	Strong yellow	Dry powdery	4 mm	Opaque	-	-
ISP3	Good	Circular	irregular	Flat	Wavy	Light greenish yellow	Moderate olive	Slimy	3 mm	Translucent	-	-
ISP4	Good	Circular	Circular	Raised	wavy	Grayish white	Pale white	Dry powdery	5 mm	Opaque	-	-
ISP5	Poor	Circular	Circular	Umbonate	filamentous	White	White	Dry powdery	4 mm	Opaque	-	-
ISP6	Good	Circular	Circular	Convex	Entire	Grayish purple	Dark purplish grey	Dry powdery	4 mm	Opaque	Dark purple	-
ISP7	Good	Circular	Circular	convex	Entire	Yellowish white	Yellowish white	Dry powdery	4 mm	Opaque	-	Brown

Sources	DTPI13
Carbon source utilization	
D-galactose	0.13
L-arabinose	0.159
D-arabinose	0.171
Cellulose	0.069
D+Galactose	0.101
D+xylose	0.094
D+glucose	0.22
Lactose	0.149
D-fructose	0.146
D-xylose	0.165
Soluble starch	0.158
Nitrogen source utilization	
L-histidine	0.136
L-asparagine	0.162
L-glutamic acid	0.16
L-tryptophan	0.188
L-valine	0.176
L-phenylalanine	0.136
L-arginine	0.168
L-threonine	0.143
L-asparatic acid	0.16
L-cysteine	0.164
Potassium nitrate	0.087
Aminoacid source utilization	
DL-alanine	+
DL-2-amino-n-butyric acid	+
L-arginine monohydrate	+
DL-asparatic acid	+
L-cysteine monohydrate	+
L-cysteine	+
L-glutamic acid	+
3-(L3,-dihydroxyphenyl)_DL	+
Glycine	+
L-histidine monohydrochloride	+
L-hydroxyproline	+
L-isoleucine	+
DL-nor-leucine	+
L-leucine	+
L-leucine monohydrochloride	+
DL-methionine	+
L-ornithine monohydrate	+
DL-phenylalanine	+
L-proline	+
DL-serine	+
DL-threonine	+
L-tryptophan	+
L-tyrosine	+
DL-valine	+
NaCl tolerance	
0.10%	0.093
1%	0.082
2%	0.055
3%	0.042
4%	0.056
5%	0.06

6%	0.062
7%	0.051
8%	0.103
9%	0.064
10%	0.102
11%	0.068
12%	0.098
15%	0.125
pH tolerance	
1	0.044
2	0.061
3	0.147
4	0.1
5	0.06
6	0.047
7	0.067
8	0.025
9	0.014
10	0.069
Temperature tolerance	
10°C	0.024
20°C	0.101
30°C	0.128
40°C	0.052
50°C	0.021
60°C	0.011
70°C	0.012
80°C	0.008
90°C	0.001
100°C	0.005

Table 1: Cultural & Physiological characteristics of *Streptomyces chartreusis* DTPI13

Properties	DTPI13
Ammonia production	+
IAA production	+
HCN production	+
OA production	1.7mm
Siderophore production	1.5mm
Phosphate solubilization	2.3mm
CMCase activity	0.3mm
Chitinolytic activity	2mm
ACC-deaminase activity	+

Table 2: Plant growth prmoting traits of isolate DTPI13 "+" indicates positive activity of isolate DTPI13

Endophytic actinobacteria molecular identification

The isolate DTPI13 was identified at the species level analyzing phylogenetic affiliation using the PCR-amplified 16S rRNA gene (Fig. 2). Using a BLAST search, the amplified 16S rRNA gene was sequenced and linked with isolate sequences from the NCBI database that were similar, and data from the NCBI database produced a number of strikingly similar sequences. *Streptomyces chartreusis* isolate DTPI13 had the highest similarity value index to *S. chartreusis*

isolate MG836688.1, followed by *S. chartreusis* isolate MK25461.1 according to the results of an NCBI-BLAST search and neighbour-joining (NJ) tree analysis. As a result, our isolate DTPI13 was identified as belonging to the *S. chartreusis* species. Our endophytic isolate *S. chartreusis* DTPI13 and MG836688.1 emerged in a same monophyletic group, according to the NJ dendrogram, whereas *S. chartreusis* isolates MK25461.1 constitute one monophyletic groupm (Fig 2). These endophytic actinobacteria DNA sequence data are available in GenBank under the accession number ON387761.

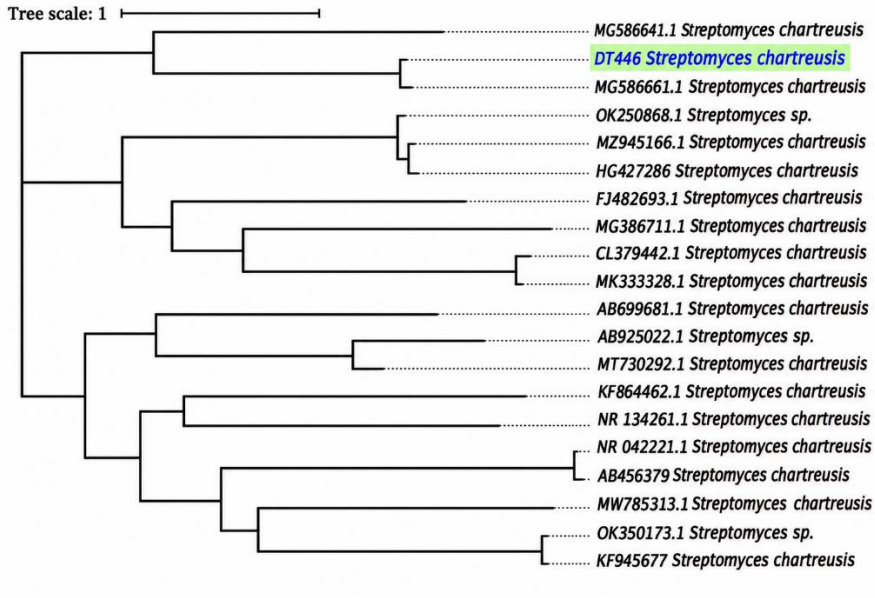


Fig 2: NJ Phylogenetic tree of *Streptomyces chartreusis* DTPI13

Conclusion

There is growing interest in the Eco physiological roles of non-*Frankia* actinobacteria that are being identified from actinorhizal plants. The actinobacteria that are non-*Frankia* have a crucial ecological role in promoting plant growth and may serve as helper bacteria to expedite the establishment of the *Frankia* symbiosis with the actinorhizal plant.

References

Ali S Z, Sandhya V and Venkateswar Rao L (2014) Isolation and characterization of drought tolerant ACC deaminase and exopolysaccharide-producing fluorescent *Pseudomonas* spp. Ann Microbiol. 64:493–502.

Anwar S, Ali B and Sajid I (2016) Screening of rhizospheric actinobacteria for various in-vitro and in-vivo plant growth promoting (PGP) traits and for agroactive compounds. Front. Microbiol. 7:1334. doi: 10.3389/fmicb.2016.01334

Azadeh B F and Meon S (2009) Molecular characterization of *Pseudomonas aeruginosa* UPM P3 from oil palm rhizosphere. American Journal of Applied Sciences, 6(11), 1915.

Baker D, Newcomb W, and Torrey J G (1980) Characterization of an ineffective actinorhizal microsymbiont, *Frankia* sp. EuI1 (Actinomycetales). Canadian Journal of Microbiology, 26(9), 1072-1089.

Bakker A W and Schippers B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. Soil Biol Biochem. 19:451–457.

Benson D R and Silvester W B (1993) Biology of *Frankia* isolates, actinobacteria symbionts of actinorhizal plants. Microbiol. Rev. 57, 2, 293–319.

Bouizgarne B and Ben Aouamar (2014) Diversity of plant associated actinobacteria in bacterial diversity in sustainable agriculture, ed D. K. Maheswari (Cham: Springer), 41–99

Cross T and Goodfellow M (1973) Taxonomy and classification of the actinobacteria. In Society for Applied Bacteriology symposium series Vol. 2, 11-112 pp.

Dworkin M and Foster J (1958) Experiments with some microorganisms which utilize ethane and hydrogen. Journal of Bacteriology, 75(5), 592-603.

Ghodhbane-Gtari F and Tisa L S (2014) Ecology and physiology of non-*Frankia* actinobacteria from actinorhizal plants. Plasticity in plant-growth-promoting and phytopathogenic bacteria, Springer, New York, NY, 27-42 pp.

Halimahtussadiyah R, Natsir M, Kurniawati D, and Utamy S P (1823) Isolation and identification of chitinolytic bacteria of pohara river of south east Sula west and the optimization production of chitinase enzyme. In *AIP Conf Proc* (Vol. 20062).

Hamedi J and Mohammadipanah F (2015) Biotechnological application and taxonomical distribution of plant growth promoting actinobacteria. Journal of Industrial Microbiology and Biotechnology, 42(2), 157-171.

Islam M R, Madhaiyan M, Deka Boruah H P, Yim W, Lee G, Saravanan VF, Fu Q, Hu H and Sa T (2009) Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. J Microbiol Biotechnol. 19(10):1213–1222. doi:10.4014/jmb.0903.3028

Johnsen H R and Krause K (2014) Cellulase activity screening using pure carboxymethylcellulose: application to soluble cellulolytic samples and to plant tissue prints. International Journal of Molecular Sciences, 15(1), 830-838.

Li Q, Chen X, Jiang Y, and Jiang C (2016) Morphological identification of actinobacteria. Actinobacteria-basics and biotechnological applications, 59-86 pp.

Marappa N, Ramachandran L, Dharumadurai D and Nooruddin T (2020) Plant growth promoting active metabolites from *Frankia* spp. of actinorhizal *Casuarina* sp. Applied Biochemistry and Biotechnology, 191(1), 74-91.

Marappa N, Dhanasekaran D and Nooruddin T (2021) Root nodule microbiome from actinorhizal *Casuarina* Plant. In Microbiome-Host Interactions, CRC Press, 295-305 pp.

Mastronunzio J E, Huang Y, and Benson D R (2009) Diminished exoproteome of *Frankia* sp. in culture and symbiosis. Applied and Environmental Microbiology, 75(21), 6721-6728.

Mondal S, and Rai V R (2019) Molecular profiling of endophytic *Streptomyces aconuresis* MH16 inhabiting *Millingtonia hortensis* Linn. and influence of different culture media on biosynthesis of antimicrobial metabolites. The Science of Nature, 106(9), 1-10 pp.

Ndeddy Aka R J and Babalola O O (2016) Effect of bacterial inoculation of isolates of Pseudomonas *aeruginosa*, *Alcaligenes feacalis* and *Bacillus subtilis* on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. Int J Phytorem. 18:200–209.

Padhi S and Tayung K (2013) Antimicrobial activity and molecular characterization of an endophytic fungus, *Quambalaria* sp. isolated from *Ipomoea carnea*. Annals of Microbiology, 63(2), 793-800 pp.

Rijavec T and Lapanje A (2016) Hydrogen cyanide in the rhizosphere: not suppressing plant pathogens, but rather regulating availability of phosphate. Front. Microbiol. 7:1785. doi: 10.3389/fmicb.2016.01785

Schwyn B and Neilands J (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 160:47–56

Shirling E T and Gottlieb D (1966) Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology, 16(3), 313-340.

Tian X L, Cao L X, Tan H M, Zeng Q G, Jia Y Y, Han W Q, and Zhou S N (2004) Study on the communities of endophytic fungi and endophytic actinobacteria from rice and their antipathogenic activities *in-vitro*. World Journal of Microbiology and Biotechnology, 20(3), 303- 309 pp.

Trujillo M E, Riesco R, Benito P and Carro L (2015) Endophytic actinobacteria and the interaction of *micromonospora* and nitrogen fixing plants. Front. Microbiol. 6:1341. doi: 10.3389/fmicb.2015.01341

Valdés M, Pérez N O, Estrada-de Los Santos P, Caballero-Mellado J, Peña-Cabriales J J, Normand P, and Hirsch a M (2005) Non- *Frankia* actinobacteria isolated from surface-sterilized roots of *Casuarina equisetifolia* fix nitrogen. Applied and Environmental Microbiology, 71(1), 460-466.

Williams S T and Cross T (1971) Chapter XI actinobacteria. In Methods in Microbiology, Academic press Vol. 4, 295-334 pp.

Zhao L, Xu Y, Sun R, Deng Z, Yang W, and Wei G (2011) Identification and characterization of the endophytic plant growth prompter *Bacillus cereus* isolate MQ23 isolated from *Sophora alopecuroides* root nodules. Brazilian Journal of Microbiology, 42, 567-575.