
Plant Growth Promoting Actinobacteria from Rhizosphere Soils of Black Pepper in Wayanad

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Abstract: Actinobacteria isolated from the rhizosphere soils of black pepper comprising both flood affected and non-flood affected areas of Wayanad district. Among different soil samples analysed, Puttad (Ptd) recorded significantly superior actinobacterial population on starch casein agar, Kenknight & Munaier's agar and actinomycetes isolation agar. Actinobacterial colonies could not be detected in any of the flooded soil samples on any medium, even at a dilution of 10^{-1} , except in Meppadi soil, which recorded a low population of 0.3×10^1 cfu g^{-1} soil. Starch casein agar is best media to isolate actinobacteria from soil samples compared to other two media. The cultural, morphological and biochemical characterization of thirty-five isolates was carried out. Further the isolates were evaluated for their plant growth promoting traits such as IAA production, nitrogen fixation, P, K and Zn solubilization. The isolates Ptd-A and Amb-C were found to be significantly superior to all other isolates, with IAA production of 15.9 g ml^{-1} and 15.38 g ml^{-1} respectively. The four isolates viz. Ptd-A, Ptd-E, Ptd-B and Ptr-A recorded significantly superior nitrogen fixation and the phosphate solubilized was significantly higher in Ptd-E, Ptd-D, Ptr-E, Ptd-A and Ptr-A, as compared to other isolates. All isolates were negative to K and Zn solubilization. Based on *in vitro* evaluations, three isolates were shortlisted (Ptd-A, Ptd-E and Ptr-A) and subjected to *in vivo* evaluation for growth promotion in black pepper (variety Panniyur 1). Rooted plants of black pepper were raised in sterile potting mixture. Bioinoculants applied at the time of planting and 45 days after planting. The PGPR Mix-1 and Organic Package of Practices Recommendations (2017) were used for comparison with the microbial inoculants along with control. In the *in-planta* experiment, biometric characters were recorded at monthly intervals, up to five months. The actinobacterial treatment, T₁: Ptd-E, T₂: Ptd-A and T₃: Ptr-A showed significant increase in shoot length, number of leaves and internode length throughout the growth period from planting to five MAP. Significantly higher root growth was observed in treatment T₂: Ptd-A, with significantly higher root volume, fresh and root weight. The potential actinobacteria were identified Ptd-A and Ptr-A as *Streptomyces* sp. and Ptd-A as *Actinobacteria bacterium* using 16S r RNA gene sequencing.

Keywords: Black Pepper, Actinobacteria, Growth Promotion, PGPR, Rhizosphere

1. Introduction

Effective farming practices, now a days, rely on extensive use of chemical fertilizers in order to enhance plant growth and yield. Excessive use of chemical fertilizers to increase production from available land has resulted in deterioration of soil quality. The use of chemical fertilizers is not only costly but also hazardous for both the environment and

humans. In addition, manufacturing of such fertilizers depletes non-renewable natural resources. An increasing demand for low-input agriculture has resulted in a greater interest in soil microorganisms which are able to enhance the plant nutrition, health and soil quality [15].

Actinomycetes are one of the major components of rhizosphere microbial populations and are useful in soil nutrient cycling as well as plant growth-promotion (PGP) [21]. Actinomycetes are the most widely distributed group of

microorganisms well known for their saprophytic survival [30]. Among them, the genus *Streptomyces* are the major sources of many biologically active compounds having antifungal, antibacterial and plant growth promoting substances [17, 29]. They mostly colonize the rhizosphere and rhizoplane and enhance plant growth is either by direct stimulation such as iron chelation, phosphate solubilization, nitrogen fixation and phytohormone production or by indirect stimulation such as suppression of plant pathogens and induction of systemic resistance in host plants [25]. The cultivable actinobacteria from soil are potentially rich sources for discovery of new actinobacterial metabolites and new actinobacterial taxa. Despite the well-documented history of *Streptomyces* in biocontrol and plant growth promotion they have been rarely exploited for their PGPR potential.

Black pepper is an important spice crop of Kerala. In India, Kerala ranks first in black pepper production, contributing about 97% of the total production and Wayanad is one of the main pepper growing tracts in Kerala, with an area of 10565 ha and production of 4136 tonnes [9]. Black pepper production in Wayanad is declining year after year mainly due to the poor soil health status, improper land management practices, with changes in climatic factors leading to the incidence of biotic and abiotic stresses.

In the present study, actinobacteria isolated from rhizosphere soils of black pepper were characterized and evaluated for PGP properties. The promising strains were also evaluated for their PGP in black pepper under *in planta*.

2. Materials and Methods

2.1. Collection of Rhizosphere Soil Samples

Rhizosphere soil samples are collected from five different locations of flood affected and non-flood affected black pepper growing areas of Wayanad districts. The soil samples were collected using quartering technique. Global Positioning System (GPS) reading of the location was recorded. About 100 g soils from each location collected in polythene bag, labelled, sealed and stored in refrigerator till further studies.

2.2. Isolation of Actinobacteria

Isolation and enumeration of actinobacteria from rhizosphere soil samples was carried out by serial dilution and plating technique [16].

Ten gram of soil sample was mixed in 90 ml sterile water and the contents were mixed thoroughly by shaking for five minutes. One ml of aliquot was transferred to 9 ml water blank and serially diluted to a final dilution of 10^{-3} and 10^{-4} for non-flood affected areas and 10^{-1} for flood affected areas. The 1 ml of suspension from respective dilutions was transferred aseptically into petri dishes. Twenty ml of molten and cooled agar of actinomycetes isolation agar, Kenknight Munaier's agar and starch casein agar media were poured into petri plates. The plates were rotated clockwise and anti-

clockwise direction and allowed to solidify. Three replicates were used for each media. Plates were incubated at 28° for 5-12 days. Number of colonies on the respective media was calculated and expressed as colony forming units per gram of soil (cfu/g).

2.3. Characterization of Isolates

2.3.1 Morphological and Cultural Characterization

The actinobacteria were grown in Kenknight's agar media at 28°C for 7 days. The characters such as colony appearance, type of aerial hyphae was observed. The colour of spore masses and diffusible pigment production were visualized. Gram's staining [13] was done and gram reaction and spore chain morphology of the isolates were observed under microscope.

2.3.2. Biochemical Characterization

i) Oxidase Test

The isolates were smeared on oxidase disc (Hi-media DD018-1VL). Appearance of violet colour within 5-10 seconds at 25-30°C indicated positive reaction. A colour change after 60 seconds or no colour change considered as negative.

ii) Catalase Test

Smear of the isolates prepared in a clean glass slides and a drop of hydrogen peroxide (3%) was added it. Cultures which immediately showed effervescence were treated as positive [31].

iii) Utilization of Sugars

Fermentation broths (glucose, fructose and sucrose) was prepared in test tubes. A Durham's tube was placed in an inverted position into the broth. The isolates were inoculated in broth and uninoculated control. The three replication is maintained and incubated for 37°C for 5 days. A change in colour from red to yellow and appearance of bubbles indicated positive for fermentation tests [7].

2.4. In Vitro Screening for Plant Growth Promoting (PGP) Activities

All the isolates were screened for plant growth promoting activities like indole acetic acid (IAA) production, nitrogen fixation, phosphate solubilization, potash solubilization and zinc solubilization under *in vitro* conditions.

2.4.1. Screening of Actinobacterial Isolates for IAA Production

The actinobacteria isolates were screened for the production of IAA [1]. All isolates were inoculated in sterile Luria-Bertani supplemented with tryptophan at the rate of 1 mg ml⁻¹. The tubes were incubated for 7 days in the dark. The cultures were centrifuged at 3000 rpm for 30 minutes and the supernatant was collected. 4 ml Salkowski reagent was added to the supernatant. The pink colour development indicated positive for IAA production. The pink colour developed test tubes were measured for optical density at 530 nm using a spectrophotometer. The OD

values were plotted on a standard graph to obtain the quantity of IAA produced by the isolates and expressed as microgram per millilitre of broth.

2.4.2. Screening of Actinobacterial Isolates for Nitrogen Fixation

The thirty-five isolates were streaked on N-free Jensen's agar media. The inoculated plates were then incubated at $28 \pm 2^\circ\text{C}$ for six days. The actinobacterial isolates which were able to grow on N free media considered as nitrogen fixers. Based on the growth in the media, nitrogen fixers were rated as high (+++), medium (++), poor (+) and no growth (-).

2.4.3. Estimation of Nitrogen Fixation

Nitrogen fixation by the thirty-five isolates were quantified by micro-Kjeldahl method [14].

$$\text{mg of N/ g of C source} = \frac{\text{TV} - \text{BV} \times \text{N} \times 0.014 \times 1000}{\text{Y}}$$

where,

TV = Titre value

BV = Blank value

N = Normality of H_2SO_4

Y = Weight of carbon source

2.4.4. Screening of Actinobacterial Isolates for Phosphate, Potassium and Zinc Solubilization

The thirty-five isolates obtained were screened for phosphate solubilization on Pikovskaya's agar media [23], potash solubilization on Aleksandrow's agar media [23], and zinc solubilization on mineral salt agar media amended with 0.1% of insoluble zinc oxide [34]. incubated for five to seven days at $28 \pm 2^\circ\text{C}$. The halo zone and colony diameter were measured after seven days of incubation. The ability to solubilize was expressed as per cent solubilization efficiency (SE)

$$\text{Solubilizing efficiency (\% SE)} = \frac{\text{SD}}{\text{CD}} \times 100$$

SD - Solubilization diameter (mm)

CD - Colony diameter (mm)

2.4.5. Estimation of Phosphate Solubilization by Actinobacterial Isolates

The isolates which showed positive reaction for phosphorus solubilization on Pikovskaya's agar containing tricalcium phosphate in the preliminary screening were further subjected to quantification of phosphorus solubilization using phosphomolybdic blue colour method [24].

2.5. Molecular Characterization

The selected actinobacteria were sent to Rajiv Gandhi Centre for Biotechnology, Trivandrum for identification by 16S rRNA sequence analysis. The sequences obtained from RGCB were compared with similar sequences from NCBI, compared using the BLAST program. The sequences of isolates were submitted to GenBank of NCBI and accession

numbers obtained.

2.6. Evaluation of Actinomycetes Strains Under Greenhouse Conditions

2.6.1. Preparation of Actinomycete Inoculum

In this experiment, the three short listed actinobacteria strains viz. Ptd-A, Ptd-E and Ptr-A were inoculated in Kenknight Munaer's broth (100 ml) and incubated for 10 days. After incubation, the broth culture was mixed with talc in the ratio of 1: 3. The formulation diluted and adjusted the cfu to 10^8 g^{-1} .

2.6.2. In Planta Evaluation of Selected Isolates in Black Pepper

The three most promising and efficient isolates were evaluated under sterile potting mixture for their growth promotion efficiency in black pepper. The experiment was conducted during May to October 2020 in the net house of Department of Agricultural Microbiology, Vellanikkara. The pot experiment was conducted with one month old rooted Panniyur 1 variety. Before planting, the uprooted plants were measured for initial growth parameters. The plants were then transplanted into polybags (16X12) filled with potting mixture (sand: soil: FYM 1: 1: 1). There were six treatments with three replications in CRD with four plants/polybag and four polybags/replications. The treatments were T₁- Ptd-E; T₂- Ptd-A; T₃- Ptr-A; T₄- PGPR Mix-1; T₅-organic POP (2017) and T₆- Control. The inoculant applied as soil drenching and foliar application 2% at the time of planting and 45 days after planting.

2.7. Statistical Analysis

Analysis of variance was done on the data collected using the statistical package WASP 2.0. [10].

3. Results and Discussion

The present study is an attempt to identify strains of potential plant growth promoting actinobacteria from flood affected and non-flood affected rhizosphere soils of black pepper in Wayanad (Table 1).

Table 1. Details of soil sample collections.

Location	Code	latitude	Longitude
Non-flooded			
Puttad	Ptd	11.57843 ⁰	76.17174 ⁰
Ambalavayal	Amb	11.61970 ⁰	76.21059 ⁰
Valat	Vlt	11.79405 ⁰	75.90547 ⁰
Pathiri	Ptr	11.8434 ⁰	76.17174 ⁰
Pulpally	Plp	11.79213 ⁰	76.16624 ⁰
Flooded			
Vellamunda	Vlm	11.7359 ⁰	75.93748 ⁰
Vythiri	Vth	11.55097 ⁰	76.04021 ⁰
Panamaram	Pnm	11.73779 ⁰	76.07398 ⁰
Adikkolly	Adk	11.78144 ⁰	76.18896 ⁰
Meppadi	Mpd	11.55514 ⁰	76.13529 ⁰

In this study, Ten soil samples consisting of five from non-flood affected and five from flood affected black

pepper gardens in Wayanad district were assessed for actinobacterial population. Enumeration was carried out on three different media viz. Kenknight Munaier's agar, Actinomycete isolation agar and starch casein agar. Different media attempted because any single medium may not be optimum for actinobacteria present in the soil. It was also expected to assess the morphology of actinobacterial colonies on different media. Among different soil samples analysed, Puttad (Ptd) recorded significantly superior actinobacterial population on all the three media. The population of Ambalavayal soil was also significantly higher on starch casein agar. Lowest population was observed in Pathiri (Ptr) sample on Kenknight & Munaier's agar and actinomycetes isolation agar (Table 2).

Table 2. Enumeration of actinobacteria from non-flooded and flooded rhizosphere soils of black pepper.

1. Non flooded areas				
Location	Code	Population of actinobacteria (10^4) cfu g ⁻¹		
		Kenknight's agar	Actinomycetes isolation agar	Starch casein agar
Puttad	P1	7 (0.84) ^a	6.67 (0.82) ^a	12 (1.08) ^a
Ambalavayal	P2	5 (0.59) ^b	3 (0.48) ^b	7 (0.84) ^a
Valat	P3	3 (0.48) ^b	2 (0.30) ^c	4.33 (0.63) ^c
Pathiri	P4	2 (0.30) ^c	1 (0.00) ^d	2.67 (0.42) ^d
Pulpally	P5	3 (0.46) ^{bc}	3 (0.48) ^b	2 (0.30) ^c

Log transformed value in parantheses.

Cultural, morphological, biochemical and physiological characters were used for characterization and identification of thirty-five isolates of actinobacteria following Bergey's Manual of Determinative Bacteriology. The isolates varied in their colony morphology, colour of aerial mycelium and pigment production. The colour of aerial mycelium varied from dark brown to light brown, white, purple or green. Colonies were circular or irregular, with the exception of Amb-A and Plt-A, which were filamentous, and Plp-A and Plp-C which were rhizoid. The elevation, form and margin of colonies varied among the isolates. Morphological characterization of the actinobacterial isolates by different parameters such as spore chain morphology, spore colour, spore shape and spore surface ornamentation are essential for classification of the actinobacteria [18]. Two isolates Vlt-K and Plp-B produced yellow water-soluble pigment and Vlt-I produced water-insoluble purple pigment. An actinobacteria produce two type of pigments water-soluble pigment or diffusible pigment and fat-soluble pigment or non-diffusible pigment.

The spore chain morphology varied among the genera and 14 isolates produced rectiflexible type of sporulating aerial hyphae ie. straight or flexuous spore chains, partly in fascicles. Six isolates each formed spira type, short chain and closed spira of sporulating aerial hyphae. Single spore, sporangia. formation and filamentous type of aerial hyphae were observed in one isolate each (Figure 1). Another study also categorized actinobacterial spore chains as rectiflexibiles, spira, retinaculiaperti, fragments, short chain, sporangia etc. as observed under the light microscope. Gram staining shows that actinobacteria are Gram positive in nature.

Actinobacterial colonies could not be detected in any of the flooded soil samples on any medium, even at a dilution of 10^{-1} , except in Meppadi soil, which recorded a low population of 0.3×10^1 cfu g⁻¹ soil.

One of the reasons for the absence of actinobacteria in flooded soils could be the limited entry of atmospheric oxygen during flooding, which could have brought about physical, chemical and biological consequences for soil habitats and species [11]. A study reported that flooding caused a decrease in the metabolically active microbial diversity, using BIOLOG microplates. Flooding led to a decline in the availability of suitable electron acceptors resulting in lower rates of organic matter decomposition [3].

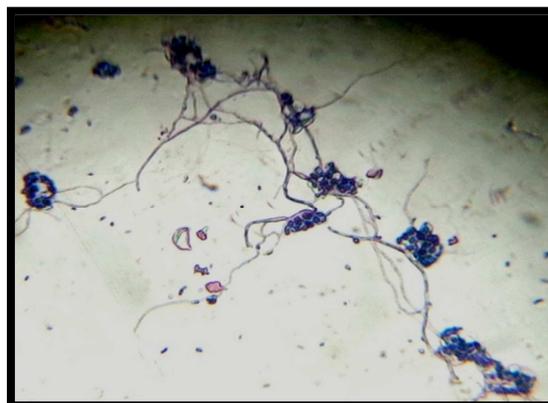


Figure 1. Spore chain morphology of Ptr-A.

The ability of 35 actinobacterial isolates to ferment sugars and produce acids from various carbohydrate sources such as glucose, sucrose and fructose was tested. Three isolates (Ptd-A, Vlt-B and Vlt-G) produced a colour change of the broth from red to yellow, without any gas formation, which indicated that they are positive to glucose fermentation test and all the 35 isolates failed to utilize fructose and sucrose. The result of the present study indicated that actinobacteria are highly non-specific in their carbon requirements. The non-specificity of actinobacteria for carbon requirements. The present study also revealed that the isolates varied in their response to catalase and oxidase. The 26 isolates were positive to catalase test --and 13 isolates produced purple colour on the oxidase disc, indicating positive reaction to oxidase test.

In the present study, 35 actinobacteria isolates were screened for IAA production on the basis of development of

pink colour in presence of tryptophan. All 35 isolates found to be positive in the preliminary screening for IAA production were used for quantitative estimation under in vitro. IAA production varied with isolates from 0.43 g ml⁻¹ to 15.9 g ml⁻¹ (Figure 2 and Table 3). The isolates Ptd-A and Amb-C were found to be significantly superior to all other isolates, with IAA production of 15.9 g ml⁻¹ and 15.38 g ml⁻¹ respectively. Similar study conducted revealed that 51% of the mangrove actinobacterial isolates produced IAA in the range of 0.21 g ml⁻¹ to 165.74 g ml⁻¹.



Figure 2. IAA production by actinobacteria.

Table 3. IAA production by actinobacterial isolates under in vitro conditions.

Sl. No.	Isolates	Concentration of IAA (µg ml ⁻¹)
1	Ptd-A	15.9 (1.32) ^a
2	Ptd-B	2.97 (0.90) ^{ijkl}
3	Ptd-C	1.19 (0.79) ^{pqr}
4	Ptd-D	3.27 (0.92) ^{hij}
5	Ptd-E	13.53 (1.27) ^b
6	Amb-A	6.35 (1.055) ^f
7	Amb-B	0.43 (0.74) st
8	Amb-C	15.38 (1.31) ^a
9	Amb-D	2.23 (0.86) ^{nm}
10	Amb-E	1.76 (0.83) ^{nop}
11	Amb-F	8.47 (1.13) ^d
12	Amb-G	1.49 (0.81) ^{op}
13	Amb-H	2.01 (0.85) ^{mno}
14	Vlt-A	3.17 (0.91) ^{hijk}
15	Vlt-B	4.14 (0.96) ^e
16	Vlt-C	2.52 (0.88) ^{klm}
17	Vlt-D	1.56 (0.82) ^{op}
18	Vlt-E	3.8 (0.94) ^h
19	Vlt-F	2.05 (0.85) ^{mno}
20	Vlt-G	2.3 (0.86) ^{lmn}
21	Vlt-H	2.55 (0.88) ^{ijklm}
22	Vlt-I	2.7 (0.89) ^{ijklm}
23	Vlt-J	0.32 (0.73) ^t
24	Vlt-K	7.18 (1.09) ^{def}
25	Vlt-L	4.35 (0.97) ^e
26	Ptr-A	11.22 (1.21) ^c
27	Ptr-B	0.86 (0.77) ^{qrs}
28	Ptr-C	3.31 (0.92) ^{hi}
29	Ptr-D	7.93 (1.11) ^{de}
30	Ptr-E	4.1 (0.96) ^e
31	Ptr-F	1.37 (0.80) ^{pqi}
32	Plp-A	6.96 (1.08) ^{ef}
33	Plp-B	0.72 (0.76) ^{rst}
34	Plp-C	1.17 (0.79) ^{pqr}
35	Mpd-A	6.64 (1.07) ^f

In the present study, thirty-five actinobacterial isolates were positive to qualitative screening for nitrogen fixation in

N-free medium. Seven isolates were further selected based on their growth on N free medium. Among the seven selected isolates, four isolates viz. Ptd-A, Ptd-E, Ptd-B and Ptr-A recorded significantly superior nitrogen fixation of 24.7 mg (by Ptd-A and Ptd-E), 23.3 mg (Ptd-B) and 22.87 mg per gram of sucrose utilized (Ptr-A) (Table 4). In a report, nitrogen fixation in roots of young pea seedlings with symbiotic *Rhizobium* sp. was observed to be promoted by *Streptomyces lydicus* strain WYEC108 [33].

Table 4. Nitrogen fixation by selected actinobacterial isolates.

Isolates	Amount of nitrogen fixed (mg of N g ⁻¹ of C utilised)
Ptd-A	24.70 ^a
Vlt-C	19.74 ^c
Ptr-C	19.97 ^{bc}
Ptd-E	24.70 ^a
Ptd-B	23.30 ^a
Amb-B	20.11 ^{bc}
Ptr-A	22.87 ^{ab}

In the present study, among 35 isolates only six were positive to preliminary screening for phosphate solubilization efficiency by the formation of clear zone on Pikovskaya's agar medium. The estimation of phosphate solubilization revealed that the amount of phosphate solubilized was significantly higher in Ptd-E (40.74 g ml⁻¹), Ptd-D (40.46 g ml⁻¹), Ptr-E (40.09 g ml⁻¹), Ptd-A (39.64 g ml⁻¹) and Ptr-A (38.89 g ml⁻¹), as compared to the isolate Ptd-B, which solubilized 31.55 g ml⁻¹ (Table 5). Various genera of actinomycetes such as *Rhodococcus*, *Arthrobacter*, *Streptomyces*, *Gordonia* and *Micromonospora* were reported to have P-solubilization potential under laboratory and glasshouse conditions [24]. The isolated 57 strains of actinobacteria and estimated phosphorus solubilization ability, which ranged from 8.7 to 118.4 mg/kg and pH from 4.0 to 5.9. Productions of various organic acids such as gluconic acid, citric acid, malic acid, lactic acid, propionic acid, oxalic and succinic acids by actinomycetes are believed to be the mechanism of their phosphate solubilisation, which also results in reduction of pH of the media [12] (Table 5).

Table 5. Quantitative estimation of Phosphate solubilization by selected isolates.

Isolates	Quantity of P- solubilized (µg ml ⁻¹)	Reduction in pH
Ptd-A	39.64 ^a	6.0
Ptd-D	40.46 ^a	5.7
Ptd-E	40.74 ^a	5.7
Ptd-B	31.55 ^b	6.1
Ptr-A	38.89 ^a	6.0
Ptr-E	40.09 ^a	5.8

In the present study, none of the isolates exhibited the ability for potassium and zinc solubilization. Nafis et al. (2019) reported that currently, little information is available on potassium solubilization by rhizospheric actinobacteria. Among 80 isolates, only two strains (*Agrobacterium tumefaciens* and *Rhizobium* sp.) produced clear halo zone around their colonies in solid medium supplemented with zinc oxide 0.1%.

The three most efficient isolates of actinobacteria were selected based on their ability to produce indole acetic acid, fix nitrogen, solubilize phosphorus, potassium and zinc. The isolates were identified by 16S rRNA sequencing. The selected actinobacterial isolates were Ptd-E (*Actinobacteria bacterium*), Ptd-A (*Streptomyces* sp.) and Ptr-A (*Streptomyces* sp.). The sequences were deposited in the NCBI data base with Gene bank Accession nos. as given in Table 6.

Table 6. Gene bank accession numbers.

Sl. No	Isolates	Location	Accession
1	Ptd-A: <i>Streptomyces</i> sp.	Puttad	MW534685
2	Ptd-E: <i>Actinobacteria bacterium</i>	Puttad	MW534690
3	Ptr-A: <i>Streptomyces</i> sp.	Pathiri	MW534694

After *in vitro* screening of isolates, the plant growth promoting activities were evaluated in sterile soil in with black pepper (variety Panniyur 1) as the test crop. The treatments included the three most efficient isolates (Ptd-E, Ptd-A and Ptr-A), the fourth treatment comprised PGPR mix-1 (commercial formulation of KAU). Organic Package of Practices Recommendations (2017) were used for comparison with the microbial inoculants along with control. Actinobacteria were made into talc-based formulation and applied at the time of planting and 45 days after planting.

In the *in-planta* experiment, biometric characters were recorded at monthly intervals, up to five months. The actinobacterial treatment, T₁: Ptd-E (*Actinobacteria bacterium*), T₂: Ptd-A (*Streptomyces* sp.) and T₃: Ptr-A (*Streptomyces* sp.) showed significant increase in shoot length,

Table 7. Effect of individual of actinobacteria in black pepper growth promotion (five months after planting).

Treatments	Fresh root wt. (g)	Dry root wt (g)	Root volume (ml)	Shoot length	No. of nodes	No of leaves
T1 – Ptd-E	3.29 ^{bc}	0.80 ^{bc}	2.59 ^b	70.82 ^a	5.85 ^{ab}	13.42 ^a
T2 – Ptd-A	4.31 ^a	1.23 ^a	3.54 ^a	77.49 ^a	6.47 ^a	15.01 ^a
T3 – Ptr-A	3.75 ^{ab}	0.92 ^b	2.69 ^b	74.75 ^a	6.32 ^a	14.04 ^a
T4 – PGPR Mix 1	2.81 ^{cd}	0.70 ^c	1.99 ^{bc}	52.53 ^b	5.83 ^{ab}	9.62 ^b
T5 – Organic POP (2017)	2.21 ^d	0.64 ^{cd}	1.75 ^{cd}	47.61 ^b	5.16 ^{bc}	8.91 ^b
T6- Control	1.52 ^e	0.51 ^d	1.25 ^d	32.25 ^c	4.73 ^c	5.47 ^c



Figure 4. Root architecture on different treatment.

Among three most potential plant growth promoting actinobacteria were selected, all actinobacterial isolates are efficient for promoting plant growth. These isolates may further be evaluated under field conditions for plant growth promotion, disease suppression and drought tolerance ability.

number of leaves and internode length throughout the growth period from planting to five MAP (Figure 3 and Table 7).



Figure 3. Effect of PGPM on plant growth.

Significantly higher root growth was observed in treatment T₂: Ptd-A (*Streptomyces* sp.), with significantly higher root volume, fresh and root weight (Figure 4 and Table 7). This isolate was found to be the most efficient IAA producer and good phosphate solubilizer also. Many studies revealed that combination of IAA production and phosphate solubilization by microbes in soil promote plant growth. *In vitro* evaluation by inoculating the roots with PGPR has revealed that many PGPR may reduce the growth rate of the primary root [8], increase the number and length of lateral roots [5; 4], and stimulate root hair elongation *in vitro* [5; 6]

The compatibility of the actinobacteria with other plant growth promoting microorganisms and chemical fertilizers may also be evaluated. In addition, expansion of local production models for commercialization of these microbial inoculants is also essential. Actinobacteria have numerous potential benefits, they can survive for longer periods in soil, in the form of spores, which may be an added advantage to develop as a biofertilizers. Only little information is available on potassium and zinc solubilizing actinobacteria and therefore further studies are required in this field. Many of the actinobacterial genera have not been exploited for agricultural, biotechnological or industrial purposes.

4. Conclusions

Most of actinobacterial isolates had multiple PGP traits. The present study revealed that the three actinobacteria from black pepper rhizosphere Ptd-A, Ptd-E and Ptr-A possessed

multiple plant growth promoting traits were found to be effective for enhancing plant growth and these isolates could be used as PGPR inoculant for black pepper cultivation. The 16S-rRNA gene sequence analysis showed Ptd-A and Ptr-A are *Streptomyces* sp. and Ptd-E are *Actinobacteria bacterium*.

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