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# Exploring the potential of actinobacteria as plant growth promoters in cowpea

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**Abstract**

The study includes isolation, characterization, and evaluation of actinobacteria for plant growth promotion in cowpea. Actinobacteria were obtained from four soil and three compost samples on starch casein agar and a total of 50 morphotypes were maintained including 21 isolates from the Department of Agricultural Microbiology repository, Kerala Agricultural University (KAU), Thrissur, Kerala. All the 50 isolates were subjected to screening for direct plant growth-promoting (PGP) activities including nitrogen fixation, phosphate, potassium and zinc solubilization, and production of indole-3-acetic acid. Indirect PGP activities including production of hydrogen cyanide, ammonia and siderophores were also tested under *in vitro* conditions. Compatibility among isolates were tested *via* cross-streak method and five actinobacterial consortia were developed for further *in planta* studies. A total of 29 actinobacterial isolates were obtained from rhizosphere soil and compost samples, with cowpea rhizosphere soil exhibiting the highest population. Based on *in vitro* screening and PGP hierarchial ranking of all the 50 isolates, 15 isolates with PGP ability were selected for further cultural, biochemical and morphological characterization. The evaluation of five compatible consortia led to significant improvement in growth and yield parameters of cowpea as compared with treatment of PGPR Mix 1 and control (*P* d" .05). The $T_2$ consortium (*Streptomyces* sp.strain DPS-7 and *Streptomyces* sp. strain Cc-5) recorded significantly the highest number of pods (23.8), number of seeds per pod (12.1), test weight (22.8 g), fresh (120.2 g) and dry weight (15.6 g) of pods, indicating their potential benefits for plant growth and yield. This research suggested actinobacterial consortia as viable biofertilizers, enhancing the growth of cowpea and contributing to environmentally sustainable agriculture.

**Keywords:** Actinobacteria, Compost, Consortia, Cowpea, Plant growth promotion

## Introduction

Cowpea (*Vigna unguiculata* L.) known as the black-eyed pea or southern pea, a member of *Leguminosae* family is an annual grain legume native from Africa, cultivated as main crop and also as an intercrop in India. It is a protein-rich crop that complements staple cereal for human and fodder for livestock. Despite being an important pulse crop, productivity has been quite low due to various biotic and abiotic constraints. This global crop encounters a number of operational constraints, including pests and diseases that limit its production and yield potentials from seedling to harvest (Asiwe, 2006). To address these challenges, sustainable and eco-friendly approaches are being explored. One promising strategy involves harnessing the plant growth-promoting (PGP) potential of actinobacteria.

The indiscriminate use of chemical inputs has been linked to detrimental consequences for human and environmental health (Glick, 2012). The usage of chemical inputs can have lasting impacts on plant evolution by altering the symbiotic relationships between plants and their microbiota, potentially influencing the co-evolution of the holobiont (Rosenberg et al., 2009) and compromising plant resilience. Therefore, there is an indispensable need for sustainable alternatives, particularly through the application of beneficial microorganisms. Many plant growth-promoting bacteria are readily available in the market, but actinobacteria have limited commercial application. The application of actinobacteria in agriculture has increased, due to their ability to promote plant growth and interact beneficially with plant rhizosphere (Yadav et al., 2018).

Actinobacteria are a group of Gram-positive bacteria, terrestrial or aquatic, having high guanine and cytosine content in their DNA. Most of the actinobacteria are

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saprophytes, ubiquitous and are one of the most diverse groups of bacteria in nature. Their nature varies from anaerobic unicellular organisms to aerobic, filamentous and spore forming lineages (Lewin et al., 2016). The morphology of actinobacteria varies from unicellular forms to complex filamentous forms, resembling fungi. Some actinobacteria produce spores by asexual reproduction. Hence many scientists hypothesize that they represent a transitional state between bacteria and fungi. Actinobacteria are among the plant growth promoting rhizobacteria (PGPR) equipped with multifunctional PGP traits and many properties beneficial to plant growth (El-Tarabily et al., 2019). These beneficial bacteria can stimulate plant growth under abiotic and biotic stress conditions *via* mechanisms such as the production of phytohormones, siderophores, ACC (1-aminocyclopropane-1-carboxylate) deaminase, exopolysaccharides, organic acids, nitrogen fixation, phosphate solubilization, various osmolytes, systemic resistance induction, etc (Khoshru et al., 2020).

Actinobacteria have been widely recognized for their plant growth-promoting (PGP) potential in crops such as tomato, wheat, rice, bean, chickpea, and pea. Passari et al. (2015) highlighted the potential of *Streptomyces* sp. Four actinobacterial strains (*Microbispora* sp. CP56, *Actinomadura* sp. CP84B, and *Streptomyces* spp. CP200B and CP21A) significantly improved chickpea growth and nodulation (Vo et al., 2021). Researchers at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have isolated and characterized PGP actinobacteria from herbal vermicomposts, demonstrating their *in vitro* PGP properties (Srinivas et al., 2021). Further investigations under controlled greenhouse and field conditions validated their effectiveness in enhancing growth and productivity in rice, sorghum, chickpea, and pigeon pea. Our study focused on isolation, screening, and characterization of actinobacteria from soil and compost samples to elucidate their potential as plant growth promoters in cowpea.

## Material and methods

*Isolation of actinobacteria from soil and compost samples*

Samples were collected from seven different sources including cowpea rhizosphere soil (CR) from Thrissur district, ginger rhizosphere soils (S) from Wayanad district, mangrove forest soil (MS) from Kadappuram (Chettuva) of Thrissur district, uncultivated soil (US) from Kerala Agricultural University (KAU) campus, Vellanikkara, Thrissur, compost (C), coir pith compost (Cc) and vermicompost (VC) from Thrissur district. Actinobacteria were isolated and enumerated from various samples using serial dilution and plating method (Johnson and Curl, 1972) on starch casein agar (HiMedia Laboratories, Mumbai, India). The Petri plates were incubated at 28 ± 2 °C for a period of five to 12 days. Pure cultures of 50 morphotypes were maintained, including 21 isolates (ACT-3, EK9K1, CS-1, WA-9, CS-10, WA-25, WA-26, WA-27, WA-28, WA-30, ACT-5, CT-2, CT-3, WA-22, DPS-5, DPS-6, DPS-7, CS-4, CS-6, CS-9, and WA-9), isolated from Black Pepper and Rice rhizosphere were obtained from the repository at the Department of Agricultural Microbiology, KAU, Vellanikkara for further studies.

*in vitro screening of actinobacteria for plant growth promoting (PGP) activities*

All the 50 actinobacterial isolates were subjected to primary and secondary screening for various direct plant growth promoting activities including production of indole-3-acetic acid (IAA), nitrogen fixation, solubilisation of phosphate, potassium and zinc and also indirect plant growth promoting activities such as production of hydrogen cyanide (HCN), ammonia and siderophores under *in vitro* conditions. Primary (qualitative) screening identified actinobacterial isolates with desired activities, whereas secondary (quantitative) screening measured the extent of these activities by quantification of the mineral solubilized and IAA produced.

*Primary and secondary screening of actinobacterial isolates for direct PGP activities*

All the 50 actinobacterial cultures were subjected to primary and secondary screening under *in vitro* conditions for direct PGP activities including nitrogen (N) fixation, solubilization of insoluble minerals including phosphate (P), potassium (K) and zinc. Production of the phytohormone, indole acetic acid (IAA) was also tested *in vitro*. Actinobacterial isolates were screened for the production of IAA (Ahmad et al., 2008). The pink colour development in Luria-Bertani (LB) broth supplemented with Tryptophan, indicated a positive reaction for IAA production and it was measured for optical density (OD) 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 µg $ml^{-1}$ of broth. Actinobacterial isolates were streaked onto N-free Jensen's agar medium, and the plates were incubated at 28 ± 2°C for six days. Isolates that exhibited growth on the nitrogen-free medium were classified as nitrogen fixers. Their ability to grow was observed and rated as follows: excellent (++++), good (+++), moderate (++), poor (+), and no growth (-). Further, Nitrogen fixation by the selected isolates was quantified by micro-Kjeldahl method (Jackson, 1973 and Bremner, 1960).

Actinobacterial isolates were screened for phosphate solubilization on Pikovskaya's agar (Nguyen et al., 1992). Isolates exhibiting potential phosphate solubilization ability in preliminary screening were further assessed for

quantitative phosphate solubilization using the phospho-molybdic blue colorimetric method (Olsen et al., 1962). The fifty isolates obtained were screened for K solubilization on Aleksandrov's agar (Nguyen et al., 1992). The quantity of potassium released by the potential actinobacteria was determined by flame photometry (Sugumaran and Janarthanam, 2007). The fifty isolates were evaluated for their ability to solubilize zinc on TRIS minimal salt agar amended with 0.1% of insoluble zinc oxide (Saravanan et al., 2004).

*Screening of actinobacterial isolates for indirect PGP activities*

Indirect PGP activities of actinobacteria including production of hydrogen cyanide (HCN), ammonia and siderophores were detected *in vitro* through primary screening. Hydrogen cyanide production was estimated using Luria-Bertani (LB) agar supplemented with glycine (4.4 g glycine/L) as per the method described by Lorck (1948). Petri plates were sealed with parafilm and incubated for 7 to 12 days at 28 ± 2°C. After incubation, development of reddish-brown colour on the filter paper indicated positive for HCN production.All actinobacterial isolates were assessed for siderophore production on Chrome Azurol Sulfonate (CAS) agar medium (Schwyn and Neilands, 1987).

After seven days of incubation, colonies that produced yellow to orange halos were positive for siderophore production. Freshly grown actinobacterial isolates were inoculated to a sterilized 4% peptone water and incubated at 28 ± 2°C for three to four days. After incubation, 0.5 ml of Nessler's reagent was added to each tube. The development of orange to the brown colour indicated ammonia production (Cappucino et al., 1992).

*Selection of potential actinobacteria for further characterization*

Promising isolates for plant growth promotion were selected based on ranking of PGP traits (Backer, 2021). Isolates of actinobacteria were arranged on the basis of their IAA production, in decreasing order. A score of 10 was allotted to each of the PGP traits and sum of the scores were considered for ranking of the isolates (Backer, 2021). Isolates having highest ranking were selected and were subjected to further characterization.

***Characterization of the selected potential acinobacterial isolates***

*Morphological and cultural characterization*

The selected actinobacteria were grown on starch casein agar media at 28°C for seven days and characterized by observing colony appearance and the types of aerial hyphae. The color of spore masses and the production of pigments were also assessed. Gram staining was performed using the method outlined by Hucker and Conn (1923). Gram reaction and spore chain morphology were observed under the compound microscope of 100x magnification.

*Biochemical characterization*

All selected isolates were subjected to biochemical characterization to test the production of oxidase and catalase and the carbohydrate source. 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.Smear of the isolates were prepared in a clean glass slides and a drop of hydrogen peroxide (3%) was added. Cultures which immediately showed effervescence were treated as positive (Taylor and Achanzar, 1972).Fermentation broth (containing glucose, sucrose and mannitol) was prepared in test tubes. Durham's tubes were placed in an inverted position in the broth. The isolates were inoculated in broth and uninoculated control was also maintained. Three replications were 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 (Cowan, 1974).

*Molecular characterization*

The identification of the best performing consortia in pot culture evaluation was carried out by 16s rRNA gene sequencing. The column Genomic DNA of actinobacteria was isolated using Nucleospin® Tissue Kit (Macherey-Nagel).One ml of five-day old culture was taken in a microfuge tube. T1 buffer (180 µl) and proteinase K (25 µl) were added to this. The mixture was placed in a water bath at 56°C till complete lysis. Then, RNA ase (100 mg/ml) was added @ five µl per tube and incubation was carried out for 5 min at room temperature. To this B3 buffer (200 µl) was added and further incubation done for 10 min at 70°C. Ethanol (100 %) was added @ 210 µl and thorough mixing was done on a vortexer. The contents were then transferred to NucleoSpin® Tissue column placed in a 2 ml collection tube. Centrifugation at 11000 x g was carried out for one min. The column was then transferred to a fresh two ml tube. Wash Buffer (BW) was used to wash the column. Washing was done again with B5 buffer (600 µl). Then the DNA in the column was eluted into a clean 1.5 ml microfuge tube, by washing with Elution Buffer (BE) @ 50 µl per column. The amplification was carried out in a polymerase chain reaction (PCR) thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). Primer combination of 16S-RS-F (forward primer) and 16S-RS-R (reverse primer) designed

by Marchesi et al. (1998) was employed for amplification of 16S rRNA sequence (Table 1).

*Table 1.* Details of primers used for 16S rRNA gene amplification

| Primer details | Sequence 5'- 3' | Length in bp |
|---|---|---|
| 16S-RS-F | CAG GCC TAA CAC ATG CAA GTC | 21 |
| 16S-RS-R | GGG CGG GTG TAT ACA AGG C | 19 |

Evaluation of quality of isolated DNA was carried out by agarose gel electrophoresis (Sambrook et al., 1989). After the separation of DNA bands using electrophoresis, the gels were visualized in a UV transilluminator and the image was taken under UV light using gel documentation system. The amplicons obtained from PCR reaction was purified and outsourced at Rajiv Gandhi Centre for Biotechnology (RGCB) in Thiruvananthapuram for sequencing, with the same combination of forward and reverse primers, as the ones employed for PCR reaction. The sequencing reaction of the PCR product was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond and Wilke, 2010). Sequence analysis and nucleotide homology of each isolates were identified through the BLASTn (basic local alignment search tool) programme of NCBI (National Centre for Biotechnology Information) (http://www.ncbi.nlm.nih.gov). The accession sharing maximum homology with the query sequence was used to identify the isolates.

*Compatibility of potential actinobacterial isolates with plant growth-promoting (PGP) activities*

The compatibility of the promising actinobacterial isolates was assessed using the *in vitro* cross-streak method. One actinobacterial isolate was streaked as a line on a starch-casein agar plate, while another test isolate was streaked perpendicularly to the first. The plates were incubated at 30°C for three days, and the growth of actinobacteria at the intersection was examined. A merger of the actinobacterial growth at this junction indicated compatibility, whereas inhibition zone indicated non-compatibility among the isolates (Al-Hussini et al., 2019).

*Preparation of talc based actinobacterial consortia*

Most efficient and promising isolates were selected for the preparation of five talc-based consortia, each containing two compatible actinobacterial isolates. Actinobacterial isolates were initially cultured separately in Kenknight's broth supplemented with potato dextrose for 7 days. Talc was sterilized at 121°C and 15 psi for two hours, then mixed with the compatible two actinobacterial cultures (in equal proportion) at a concentration of $10^8$CFUml$^{-1}$in a 1:2.5 ratio.

*In planta* evaluation of actinobacterial consortia for growth promotion in cowpea

The five actinobacterial consortia were assessed under sterile conditions for their efficacy in growth promotion of cowpea. The experiment was carried out from June to August 2024, using completely randomized design (CRD). The experiment consisted of seven treatments with three replicates, and each replicate had three pots. The first five treatments comprised the selected consortia, whereas the sixth and seventh treatments included PGPR Mix 1 from KAU and an uninoculated control, respectively. The inoculum was applied @ 10 g per polybag under each treatment. Sterile potting mixture was used for the experiment. Seeds of Bhagyalakshmy variety were treated with the KAU culture of *Rhizobium* sp. (strain Rh4), with three to four seeds sown in each pot. The soil was inoculated with the talc based actinobacterial consortia twice, initially one week after sowing and again one month after the first application. The population of actinobacteria in the potting mixture was evaluated at 30, 60 and 90 days after sowing (DAS). Observations on biometric parameters such as plant height, number of leaves, number of branches, days to flowering, number of effective nodules per plant were recorded at monthly intervals for three months after sowing. Fresh and dry weight of shoots, root parameters like fresh and dry weight of roots (g per plant) and root volume were recorded at 90 DAS. Yield parameters such as number of pods per plant, number of seeds per pod, test weight (100 seeds), fresh and dry weight of pods (g per pod) were observed after 90 DAS. Initial and final nutrient (N, P and K in kg ha$^{-1}$) profile of the potting mixture and nutrient status of plants after the experiment were recorded.

*Statistical analysis*

Analysis of variance (ANOVA) suitable to CRD was performed on the collected data using GRAPES version 1.0.0 (Gopinath et al., 2021) statistical software.

## Results and discussion

*Isolation of actinobacteria*

Modern agriculture needs sustainable practices to increase crop yields, boost production, and enhance soil fertility (Yasari et al., 2009). Actinobacteria can function as bio-inoculants and biopesticides, serving as an eco-friendly alternative to chemical fertilizers, by aiding crops in tolerating stress factors such as temperature, pH, salinity, and drought (Cheng et al., 2018). Hence the present study was envisaged on the isolation, screening, and characterization of actinobacteria from rhizosphere soil,

uncultivated soil, mangrove forest soil and compost samples to assess their potential as promoters of plant growth in cowpea. Among the various soil and compost samples analysed, cowpea rhizosphere soil (CR) recorded significantly superior actinobacterial population on starch casein agar, with a population of 42.9 x $10^6$ cfu $g^{-1}$,while coirpith compost (Cc) recorded the population of 19.0 x $10^6$ cfu $g^{-1}$. The lowest population was recorded in mangrove forest soil (MS) and ginger rhizosphere soil (S) with a population of 1.1x$10^6$ cfu $g^{-1}$ and 1.2x$10^6$ cfu $g^{-1}$. In contrast, coir pith compost and ginger rhizosphere soil reported a significantly higher number of morphotypes (7), while mangrove forest soil recorded the lowest number of morphotypes (1) (Fig.1).

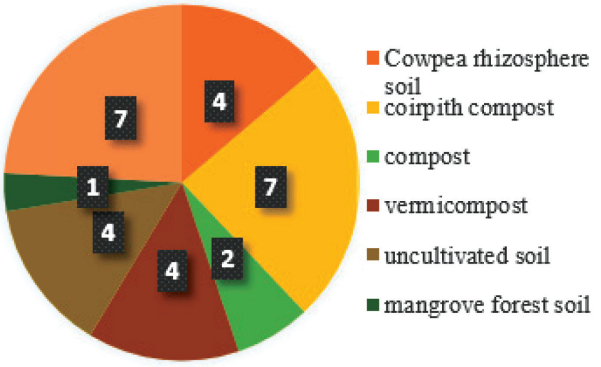



*Figure 1:* Number of morphotypes obtained from various samples

Numerous studies support the findings of this research. Khamna et al. (2010) isolated 270 *Streptomyces* spp. from the rhizosphere soils of 14 Thai medicinal plants and reported that *Streptomyces* CMU-H009, isolated from lemongrass (*Cymbopogon citratus*) rhizosphere soil, was found to be highly effective in synthesizing indole-3-acetic acid (IAA). Gopalakrishnan et al. (2012) reported that actinobacterial isolates obtained from herbal vermicompost can be employed for the biological control of *Fusarium* wilt of chickpea. Sreevidya et al. (2016) isolated and characterized actinobacteria with plant growth-promotion, from the rhizosphere of chickpea. Actinobacteria are susceptible to low pH (ideal pH range: 6.5-8.0) and acidity, as well as wet conditions. These are mesophilic organisms that thrive at 25-30°C, whereas some species that are frequently found in compost and manures are thermophilic and grow at 55-65°C (e.g. *Thermoactinomycetes*, *Streptomyces*) (Bhatti et al.,2017). Meanwhile, Woliñska et al. (2019) reported that greater abundance of actinobacteria was observed in non-cultivated soils relative to the cultivated soils. Moreover, it was indicated that the actinobacterial diversity depended on both the soil genesis and the land use; however, this effect directly depended on the particular family and genera. Nalini et al. (2020) reported that among the 40 actinobacterial isolates isolated from the different rhizosphere soil samples of legume crop, cowpea was rich in the actinobacteria population and starch casein agar proved to be the best medium for their enumeration. Law et al. (2023) reported a novel strain, *Streptomycesgriseiviridis* MUM 136J$^T$ from a mangrove forest soil in Malaysia. Recently, Uesugi et al. (2024) isolated and characterized actinobacteria from industrial composting soil of oil palm (*Elaeis guineensis*) in the municipality of Igarapé-Açu, Pará.

*in vitro screening of actinobacterial isolates for plant growth promoting (PGP) traits*

Actinobacteria are known for synthesizing large amounts of phytohormones that enhance plant growth. Additionally, they play a crucial role in mobilizing nutrients like phosphate, zinc, and potassium in soils deficient in these micronutrients, thus significantly contributing to plant growth promotion (Mitra et al., 2022). Therefore, it can be regarded as plant biofertilizers (Franco-Correa and Chavarro-Anzola, 2016). In the present study, 50 actinobacterial morphotypes were initially screened *in vitro*, for the presence of direct plant growth-promoting traits like IAA production, $N_2$ fixation and also the solubilization of phosphate, potassium and zinc. Subsequently, secondary screening was conducted to assess the extent of production of the plant growth promoting substance. *In vitro* primary screening for IAA production revealed that among 50 isolates, four (ACT-3, Cc-5, US-3 and VC-5) were categorized as excellent, three (WA-25, C-1 and VC-4) as good, two (EK9K1, WA-25) as moderate, 10 as low IAA producers and 31 isolates as no IAA producers based on the intensity of pink colour development upon addition of Salkowski reagent. Secondary screening of IAA producers revealed that the amount of IAA production was ranged between 11.3 and 90.9µg $ml^{-1}$. The isolate ACT-3 (90.9µg $ml^{-1}$)was found to be significantly superior to all other isolates. There are many reports which demonstrated the ability of actinobacteria to produce IAA and thus promote plant growth (Solans et al., 2011; Dochhil et al., 2013). Suksaaid et al. (2017) reported that 51% of the mangrove actinobacterial isolates produced IAA in the range of 0.2 µg $ml^{-1}$ to 165.7 µg $ml^{-1}$ .

Nitrogen fixation by microbes plays a vital role in promoting plant growth and increasing yield. Franche et al. (2009) emphasized that nitrogen is one of the most limiting nutrients for plant development. Although atmospheric nitrogen makes up approximately 78% of the Earth's atmosphere, it remains unavailable to plants, animals, and the majority of microorganisms in its diatomic form. Primary screening of actinobacteria for Nitrogen fixation demonstrated that among 50 isolates, 48 isolates were predicted to fix atmospheric nitrogen where 15 isolates were strong nitrogen fixers. Four

isolates *viz.* DPS-7, MS-3(1), Cc-4 and CR-3 recorded significantly superior nitrogen fixation. There are numerous evidences of actinobacteria involved in nitrogen fixation. The genus *Frankia* are widespread endophytic actinobacteria symbiotically associated with plant roots and fix atmospheric nitrogen for host plants (Benson and Silvester, 1993). Inoculation of pea seedlings with *Streptomyces lydicus* strain WYEC108 along with *Rhizobium* sp. enhanced nitrogen fixation in the roots (Tokala et al., 2002). According to Wang et al. (2019) *Streptomyces chartreusis* WZS021 has the ability to carry out nitrogen fixation, resulting in a 30 per cent increase in nitrogen content in sugarcane shoots and a 36 per cent increase in roots.

In agriculture, biological phosphate solubilization serves as an important alternative to the use of natural phosphate minerals, enhancing nutrient uptake efficiency. In the current study, sixteen isolates exhibited phosphate solubilisation on Pikovskaya's media and the amount of phosphate solubilized ranged from 89.5 to 95.1 to µg $ml^{-1}$, as observed in the isolates WA-9 and Cc-1 These results are supported by many studies, Anwar et al. (2016), screened actinobacterial isolates for their phosphate solubilizing abilities and revealed that *Streptomyces* sp. WA-1 produced the highest soluble phosphate concentration (72.1 µg $ml^{-1}$), followed by *S. djakartensis* TB-4 (70.3 µg $ml^{-1}$). Potassium (K) is recognized as the third essential macronutrient required for plant growth and development. Potassium-solubilizing micro-organisms (KSM) facilitate the release of potassium from insoluble mineral sources by converting it into a soluble form which can be readily absorbed by plants. In the present investigation among 50 isolates, potassium solubilization was observed in two isolates (US3 and VC5) (Fig. 2). Etesami et al. (2017) reported that only 5% of potassium solubilizing bacteria available are actinobacteria. Archana (2007) isolated 30 potassium-solubilizing bacterial strains from soils in the Belgaum and Dharwad districts of Karnataka, using mica as an insoluble potassium source and potassium solubilization by the actinobacterial isolates varied from 2.4 to 44.4 µg $ml^{-1}$.

Zinc is an essential micronutrient required for plant growth and development. However, when applied in its inorganic form, a significant amount of zinc becomes immobilized in the soil, rendering it insoluble and unavailable for plant uptake. Zinc-solubilizing bacteria are regarded as effective biological agents for enhancing zinc availability, offering an alternative to conventional zinc-based fertilizers (Praveen et al., 2013). In the present study study, none of the isolates showed the ability for zinc solubilisation on TRIS minimal salt agar medium supplemented with 0.1% insoluble zinc oxide under *in vitro* conditions. Limited studies have been

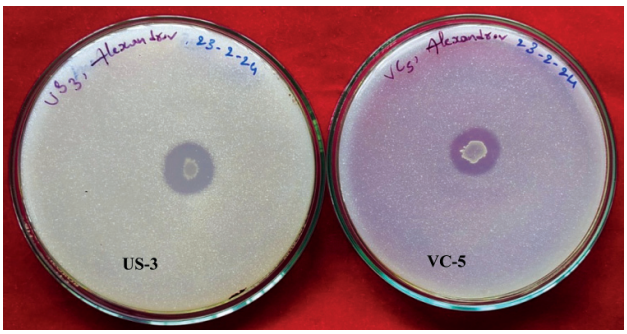



*Figure 2.* Primary screening of actinobacterial isolates for potassium solubilization

conducted on the solubilization of zinc by actinomycetes. However, Patel and Thakker (2020) found that *Streptomyces nanhaiensis* strain YM4 was capable of solubilizing zinc, with a concentration of 41.16 ppm.

A total of twenty-nine actinobacterial isolates exhibiting at least one direct plant growth-promoting (PGP) trait were selected and assessed for their ability to enhance plant growth through IAA production, nitrogen fixation, and solubilization of phosphate and potassium (Backer, 2021). Based on their efficiency, the top fifteen isolates were identified as the most promising and were chosen for further characterization (Table 2).

*Selection of efficient PGP actinobacteria for further characterization and in planta evaluation*

The isolates with first 15 ranks (Cc-5, US-3, Cc-4, Cc-6, EK9K1, DPS-5, WA-26, Cc-2, WA-27, DPS-7, CS-1, C-2, WA-30 and CS-10) were selected as the most efficient ones after ranking the isolates and were used for further characterization of actinobacteria followed by *in planta* evaluation.

*Characterization of actinobacterial isolates*

Actinobacterial isolates were characterized morphologically using Gram staining, which confirmed that they were Gram-positive and exhibited a filamentous structure.The microscopic characterization based on substrate and aerial mycelium and spore chain morphology is a very useful tool in the identification of actinobacteria (Burkholder etal.,1954). While observing the spore chain morphology, it was observed that among the15 selected isolates, five were rectiflexibles, three formed shortchain, two were flexuous, two were filamentous and three produced spira spore chains.Cultural and morphological characteristics of actinobacteria are presented in Table 3 and Fig. 3. Li et al. (2016) reported that actinobacteria produce two type of pigments water-soluble or diffusible pigment and fat-soluble or non-diffusible pigment. In our study, diffusible pigment production was observed in Cc-1 and Cc-6 (Fig. 4) and non-diffusible

*Table 2.* Ranking of plant growth promoting isolates of actinobacteria based on efficiency under *in vitro* conditions

| Actinobacterial isolate | Production of IAA (µg ml$^{-1}$) | Quantity of nitrogen fixed (mg of N g$^{-1}$ of C utilised) | Amount of P solubilized (µg ml$^{-1}$) | Amount of K solubilized (µg ml$^{-1}$) | Amount of Zn solubilized (µg ml$^{-1}$) | Sum of scores (x10) | Rank allotted |
|---|---|---|---|---|---|---|---|
| Act-3 | 90.9$^{a}$ | - | - | - | - | 909.0 | 16 |
| Cc-5 | 53.8$^{b}$ | - | 91.8$^{f}$ | - | - | 1457.0 | 1 |
| US-3 | 28.0$^{c}$ | - | 93.0$^{d}$ | 3.8$^{a}$ | - | 1250.3 | 2 |
| VC-5 | 25.5$^{d}$ | - | - | 3.5$^{b}$ | - | 290.3 | 18 |
| CR-5 | 17.6$^{e}$ | - | - | - | - | 176.6 | 19 |
| C-1 | 16.6$^{f}$ | - | - | - | - | 166.8 | 20 |
| VC-4 | 15.6$^{g}$ | - | - | - | - | 156.3 | 21 |
| WA-25 | 14.4$^{h}$ | - | - | - | - | 144.7 | 24 |
| EK9K1 | 11.3$^{i}$ | - | 94.2$^{b}$ | - | - | 1055.5 | 5 |
| DPS-7 | - | 15.4$^{a}$ | 91.8$^{f}$ | - | - | 934.2 | 11 |
| Cc-4 | - | 15.3$^{a}$ | 92.1$^{e}$ | - | - | 1075.0 | 3 |
| MS-3(1) | - | 15.3$^{a}$ | - | - | - | 153.9 | 22 |
| CR-3 | - | 15.3$^{a}$ | - | - | - | 153.6 | 23 |
| Cc-6 | - | 12.8$^{b}$ | 93.8$^{c}$ | - | - | 1067.2 | 4 |
| DPS-5 | - | 12.8$^{b}$ | 91.6$^{g}$ | - | - | 1044.8 | 6 |
| S1-3 | - | 10.2$^{c}$ | - | - | - | 102.4 | 25 |
| S4-2 | - | 5.1$^{d}$ | - | - | - | 51.3 | 27 |
| Cc-3 | - | 5.1$^{d}$ | - | - | - | 51.3 | 28 |
| S3-2 | - | 5.1$^{d}$ | - | - | - | 51.3 | 26 |
| WA-26 | - | 5.1$^{d}$ | 90.8$^{j}$ | - | - | 959.3 | 7 |
| Cc-2 | - | 5.1$^{d}$ | 90.6$^{j}$ | - | - | 958.0 | 8 |
| WA-27 | - | 2.5$^{e}$ | 91.1$^{i}$ | - | - | 942.2 | 10 |
| S3-1 | - | 2.5$^{e}$ | - | - | - | 25.7 | 29 |
| Cc-1 | - | - | 95.1$^{a}$ | - | - | 951.0 | 9 |
| CS-1 | - | - | 92.1$^{e}$ | - | - | 921.2 | 12 |
| C-2 | - | - | 91.8$^{f}$ | - | - | 918.7 | 13 |
| WA-30 | - | - | 91.4$^{h}$ | - | - | 914.1 | 14 |
| CS-10 | - | - | 91.1$^{i}$ | - | - | 911.6 | 15 |
| WA-9 | - | - | 89.5$^{k}$ | - | - | 895.5 | 17 |

*Treatments with same letters are not significantly different (-) : Absent

pigment production was observed in C-2. However, Thampi and Bhai (2017) stated that colony morphology is influenced by the type of media used, and therefore, cannot be considered as a fundamental criterion for identifying microbes. Biochemical characterization of the isolates are presented in Table 4. In a similar study, Vyawahare et al. (2013) found that nine *Streptomyces* strains tested positive for both catalase and oxidase activity.

*Compatibility of potential actinobacteria with PGP activities*

To develop the consortia formulation of PGP actinobacteria, compatibility among 15 selected isolates was tested by using the cross-streak method. All possible combinations of nine promising isolates were tested and the results revealed that among 15 isolates, only eight isolates exhibited compatibility. From this, five best combinations of two compatible isolates (Cc-5 and Cc-6; DPS-7 and Cc-5; DPS-7 and Cc-4; DPS-5

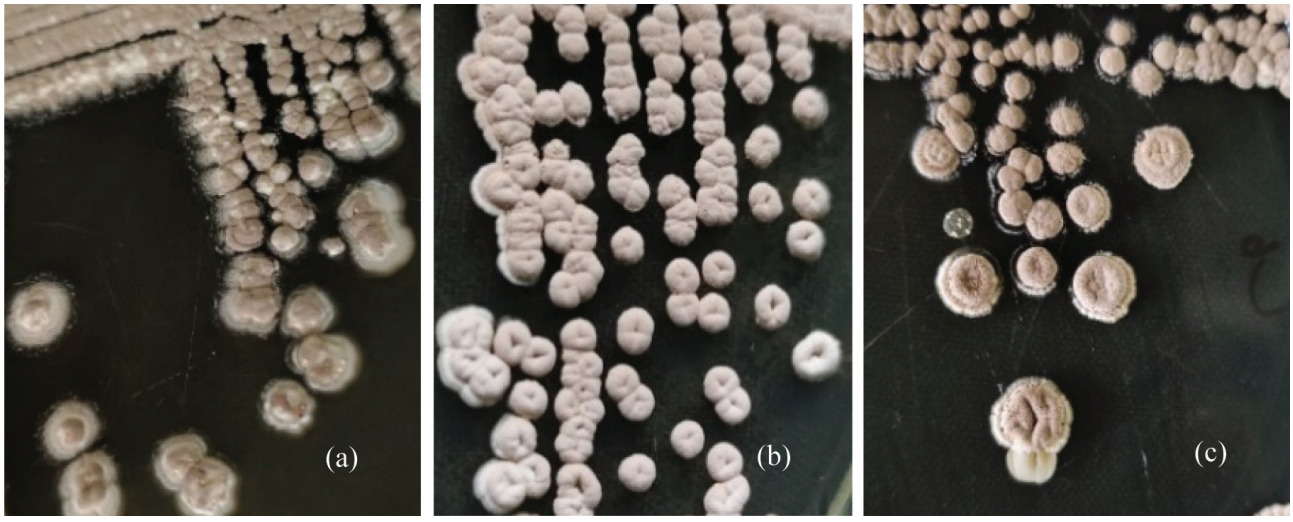



*Figure 3.* Colony morphology of isolates. (a) DPS-7, (b) Cc-4 (c) Cc-6

*Table 3.* Morphological and cultural characterization of isolates

| Sl. No. | Actinobacterial isolates | Colony colour | Elevation | Form | Margin | Colour of aerial mycelium | Gram reaction | Pigment produced | Spore chain morphology |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Cc-5 | Grey | Umbonate | Circular | Entire | Grey | + | - | Short chain |
| 2 | US-3 | Creamish white | Umbonate | Circular | Curled | Yellow | + | - | Flexuous |
| 3 | Cc-4 | Creamish white | Raised | Circular | Entire | White | + | - | Rectiflexibles |
| 4 | Cc-6 | Off-white | Umbonate | Circular | Entire | White | + | Light yellow (Diffusible) | Filamentous |
| 5 | EK9K1 | Whitish grey | Convex | Circular | Filiform | White | + | - | Rectiflexibles |
| 6 | DPS-5 | White | Umbonate | Concentric ring | Entire | White | + | - | Short chain |
| 7 | WA-26 | White | Umbonate | Irregular | Curled | White | + | - | Spira |
| 8 | Cc-2 | Grey | Flat | Circular | Filiform | Grey | + | - | Rectiflexibles |
| 9 | Cc-1 | Light brown | Raised | Circular | Entire | Brown | + | Yellow (Diffusible) | Flexuous |
| 10 | WA-27 | Grey | Umbonate | Circular | Filiform | Grey | + | - | Filamentous |
| 11 | DPS-7 | Grey | Raised | Irregular | Entire | Grey | + | - | Rectiflexibles |
| 12 | CS-1 | Creamish white | Umbonate | Circular | Filiform | White | + | - | Spira |
| 13 | C-2 | Pinkish white | Slightly raised | Circular | Filiform | White | + | Red (Non-diffusible) | Short chain |
| 14 | WA-30 | Off white | Slightly raised | Circular | Entire | White | + | - | Spira |
| 15 | CS-10 | Grey | Raised | Circular | Entire | Grey | + | - | Rectiflexibles |

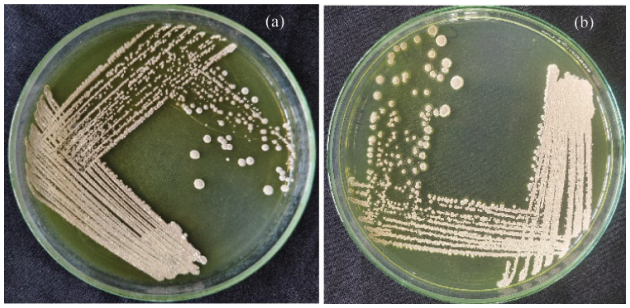



*Figure 4.* Diffusible pigment production by actinobacterial isolates (a) Cc-1 and (b) Cc-6

*Table 4.* Characterization of isolates based on biochemical tests

| Sl. No. | Actinobacterial isolates | Catalase | Oxidase | Sugar utilization | | |
|---|---|---|---|---|---|---|
| | | | | Glucose | Sucrose | Mannitol |
| 1 | Cc-5 | + | + | - | - | - |
| 2 | US-3 | + | - | - | + | - |
| 3 | Cc-4 | + | - | - | - | - |
| 4 | Cc-6 | + | - | - | - | + |
| 5 | EK9K1 | + | - | - | - | - |
| 6 | DPS-5 | + | - | - | - | - |
| 7 | WA-26 | + | + | - | - | - |
| 8 | Cc-2 | + | + | - | - | - |
| 9 | Cc-1 | + | - | - | - | - |
| 10 | WA-27 | + | + | - | - | - |
| 11 | DPS-7 | + | + | - | - | - |
| 12 | CS-1 | + | - | - | - | - |
| 13 | C-2 | + | + | - | - | - |
| 14 | WA-30 | - | + | - | - | - |
| 15 | CS-10 | - | - | - | - | - |
| | Total | 13 | 7 | 0 | 1 | 1 |

+ WA-26; WA-27 and Cc-2) were selected for the preparation of talc-based consortia for further *in planta* evaluation.

*In planta evaluation of selected actinobacterial consortia for growth promotion in cowpea*

A pot culture experiment was conducted to evaluate the effect of five actinobacterial consortia in enchancing cowpea growth and yield. The population of actinobacteria ranged from 1.1 x$10^6$ cfu $g^{-1}$ to 6.0 x$10^6$ cfu $g^{-1}$ and $T_2$ and $T_5$ were statistically significant at all the intervals. No actinobacteria could be detected in the treatments $T_6$ (PGPR Mix 1 of KAU) and $T_7$ (Uninoculated control) at 30, 60 and 90 DAS (Table 5).

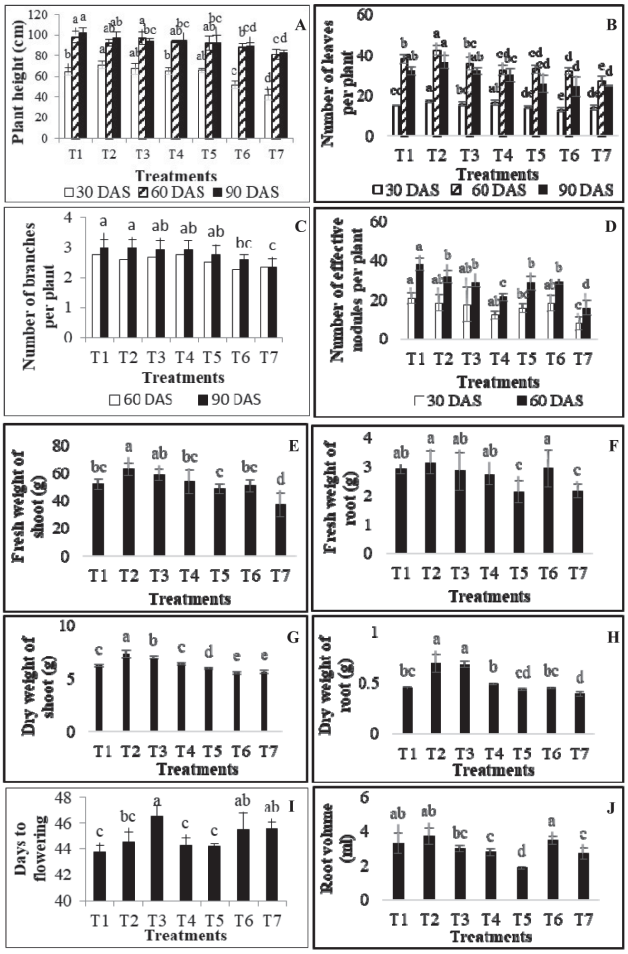
Actinobacterial inoculation significantly improved all the growth and yield parameters compared to the control treatment (*P* d" .05). At 90 DAS, significantly higher plant height was observed in $T_1$, $T_2$, $T_3$, and $T_4$. Number of leaves per plant was significantly higher in $T_1$, $T_2$, and $T_3$, while $T_2$ had highest number of branches. $T_2$ and $T_3$ recorded significantly higher fresh shoot weight. $T_1$, $T_2$, $T_3$, and $T_4$ recorded higher fresh root weight, while $T_2$ and $T_3$ recorded significantly higher dry root weight. Significantly higher root volume was observed in $T_1$, $T_2$, and $T_6$. Time taken for flowering varied significantly among

*Table 5.* Population of actinobacteria in the rhizosphere of cowpea at various intervals (x$10^6$ cfu $g^{-1}$)

| Sl. No. | Treatments | Actinobacterial population in potting mixture (x$10^6$ cfu $g^{-1}$) | | |
|---|---|---|---|---|
| | | 30 DAS | 60 DAS | 90 DAS |
| 1 | $T_1$: Consortium 1 (Cc-5 + Cc-6) | 5.7 (0.755)$^a$ | 4.4 (0.650)$^b$ | 4.1 (0.6127)$^a$ |
| 2 | $T_2$: Consortium 2 (DPS-7 + Cc-5) | 6.0 (0.781)$^a$ | 5.6 (0.748)$^a$ | 4.2 (0.630)$^a$ |
| 3 | $T_3$: Consortium 3 (Cc-4 + DPS-7) | 4.3 (0.633)$^b$ | 3.7 (0.568)$^c$ | 3.1 (0.501)$^b$ |
| 4 | $T_4$: Consortium 4 (WA-26 + DPS-5) | 3.6 (0.562)$^b$ | 3.4 (0.534)$^c$ | 2.7 (0.439)$^{bc}$ |
| 5 | $T_5$: Consortium 5 (WA-27 + Cc-2) | 5.7 (0.757)$^a$ | 5.2 (0.722)$^a$ | 2.5 (0.397)$^c$ |
| 6 | $T_6$: PGPR Mix-1 of KAU | 0 | 0 | 0 |
| 7 | $T_7$: Control (Uninoculated) | 0 | 0 | 0 |
| | CD @ 0.05 | 0.080 | 0.087 | 0.061 |

Population of actinobacteria in the rhizosphere of cowpea at various intervals (x$10^6$ cfu $g^{-1}$)

Log transformed values are given in parentheses and means followed by common letter(s) do not significantly differ




**$T_1$:** Consortium 1 (DPS-7 + Cc-5); **$T_2$**: Consortium 2 (WA-27 + Cc-2); **$T_3$**: Consortium 3 (Cc-5 + Cc-6); **$T_4$**: Consortium 4 (WA-26 + DPS-5); **$T_5$**: Consortium 5 (Cc-4 + DPS-7); **$T_6$**: PGPR Mix 1 of KAU; **$T_7$**: Control (uninoculated)

*Figure 5***.** Effect of actinobacterial consortia on biometric parameters (A-J) in cowpea. Values were expressed as mean ± standard error. Different letters indicate significant differences at *P* d" .05 and treatments with same letters are not significantly different. A: plant height; B: number of leaves per plant; C: number of branches per plant: D: number of effective nodules per plant: E: fresh weight of shoot; fresh weight of root; G: dry weight of shoot; H: dry weight of root: I: days to flowering; J: root volume.

treatments. The time period until first blooming varied from 43.7 days (observed in$T_1$)to 46.5days (in treatment $T_3$). Destructive sampling (Three plants per treatment) was conducted at 60 DAS and at the time of harvest (90DAS) to record the number of nodules per plant. At 60 DAS, $T_1$ recorded maximum number of nodules (37.9) and $T_7$ exhibited significantly lower nodule number (15.6 per plant) (Fig. 5). Thenappan et al. (2024) reported that two strains of *Streptomyces* spp., obtained from rice rhizosphere enhanced root surface area and the volume of soil traversed by roots. This resulted in improved nutrient uptake and significant growth-promoting effects in rice.

Meanwhile, yield parameters such as number of pods (33.8), number of seeds per pod (12.1), test weight (22.8 g), fresh and dry weight of pods (192.3 g and 20.6 g) were also superior in $T_2$, indicating their potential benefits for plant growth and yield (Figure 6). Nutrient analysis of the plant samples after harvest revealed that the amount of total nitrogen in plants was significantly higher in $T_2$ (0.3550 %), and $T_3$ (0.3350 %), which were statistically on par (Table 6).

*Table 6*. Effect of actinobacterial isolates on nutrient status (Final) of plants under pot culture

| Sl. No. | Treatments | Total nutrient content (%) | | |
|---|---|---|---|---|
| | | Nitrogen | Phosphorus | Potassium |
| 1 | $T_1$: Consortium 1 (Cc-5 + Cc-6) | $0.3250^{b}$ | $0.0080^{a}$ | $0.0045^{ab}$ |
| 2 | $T_2$: Consortium 2 (DPS-7 + Cc-5) | $0.3550^{a}$ | $0.0076^{a}$ | $0.0051^{a}$ |
| 3 | $T_3$: Consortium 3 (Cc-4 + DPS-7) | $0.3350^{ab}$ | $0.0070^{ab}$ | $0.0040^{b}$ |
| 4 | $T_4$: Consortium 4 (WA-26 + DPS-5) | $0.3100^{bcd}$ | $0.0073^{a}$ | $0.0044^{ab}$ |
| 5 | $T_5$: Consortium 5 (WA-27 + Cc-2) | $0.2950^{cd}$ | $0.0069^{ab}$ | $0.0038^{bc}$ |
| 6 | $T_6$: PGPR Mix-1 of KAU | $0.3200^{bc}$ | $0.0075^{a}$ | $0.0042^{b}$ |
| 7 | $T_7$: Control (Uninoculated) | $0.2850^{d}$ | $0.0058^{b}$ | $0.0033^{c}$ |
| | CD @ 0.05 | 250.6 | 11.7 | 6.9 |

Values succeeded by the same letter are not significantly different, according to DMRT

The total phosphorus content in plants was significantly higher in all the treatments which received actinobacterial consortium or PGPR Mix-1 and $T_7$ (Uninoculated control) recorded a lower content of phosphorus (0.0058 %). Similarly, $T_1$, $T_2$ and $T_4$ recorded significantly higher content of total potassium (0.0045 %,0.0051 % and 0.0044 % respectively), as compared to the other treatments. Overall, application of actinobacteria improved nutrient uptake by plants, in comparison with uninoculated control. Recent reports indicate that *Streptomycescoelicoflavus* inoculated in mangrove soil enhanced available Pinsediments, and also in roots and shoots, when compared to uninoculated control (El-Tarabily et al., 2021). Post-experimental soil analysis also revealed a significant increase in available phosphorus content in the potting mixture (Table 7).

This finding supports the results of several earlier studies. A study employing the "ragdoll" method investigated the impact of four *Streptomyces* spp. on chickpea seedling growth, revealed VAI-7 as the most effective strain in enhancing shoot and root length. Under field conditions, the *Streptomyces* spp. increased nodule number over un-inoculated control demonstrating a direct proof for enhancing nitrogen fixation (Table 2). The *Streptomyces* strains used in the study exhibited increase in agronomic properties such as the shoot weight, leaf weight, leaf area, plant height, grain yield and stover yield over the un-inoculated control

*Table 7.* Nutrient content (kg ha$^{-1}$) of potting mixture as influenced by actinobacterial consortia

| Sl. No. | Treatments | Available N | Available P | Available K |
|---|---|---|---|---|
| 1 | $T_1$: Consortium 1 (Cc-5 + Cc-6) | 210.2$^{d}$ | 551.7$^{d}$ | 359.0$^{d}$ |
| 2 | $T_2$: Consortium 2 (DPS-7 + Cc-5) | 322.7$^{a}$ | 794.5$^{a}$ | 533.7$^{a}$ |
| 3 | $T_3$: Consortium 3 (Cc-4 + DPS-7) | 220.2$^{d}$ | 685.7$^{b}$ | 396.2$^{b}$ |
| 4 | $T_4$: Consortium 4 (WA-26 + DPS-5) | 254.7$^{c}$ | 577.5$^{c}$ | 326.7$^{c}$ |
| 5 | $T_5$: Consortium 5 (WA-27 + Cc-2) | 206.2$^{d}$ | 533.5$^{c}$ | 378.2$^{c}$ |
| 6 | $T_6$: PGPR Mix-1 of KAU | 290.2$^{b}$ | 683.5$^{b}$ | 404.0$^{b}$ |
| 7 | $T_7$: Control (Uninoculated) | 194.5$^{e}$ | 503.0$^{f}$ | 330.2$^{c}$ |
| | CD @ 0.05 | 22.79 | 14.55 | 11.04 |
| | Initial soil testing value | 189.0 | 498.9 | 327.9 |

Values succeeded by the same letter are not significantly different, according to DMR

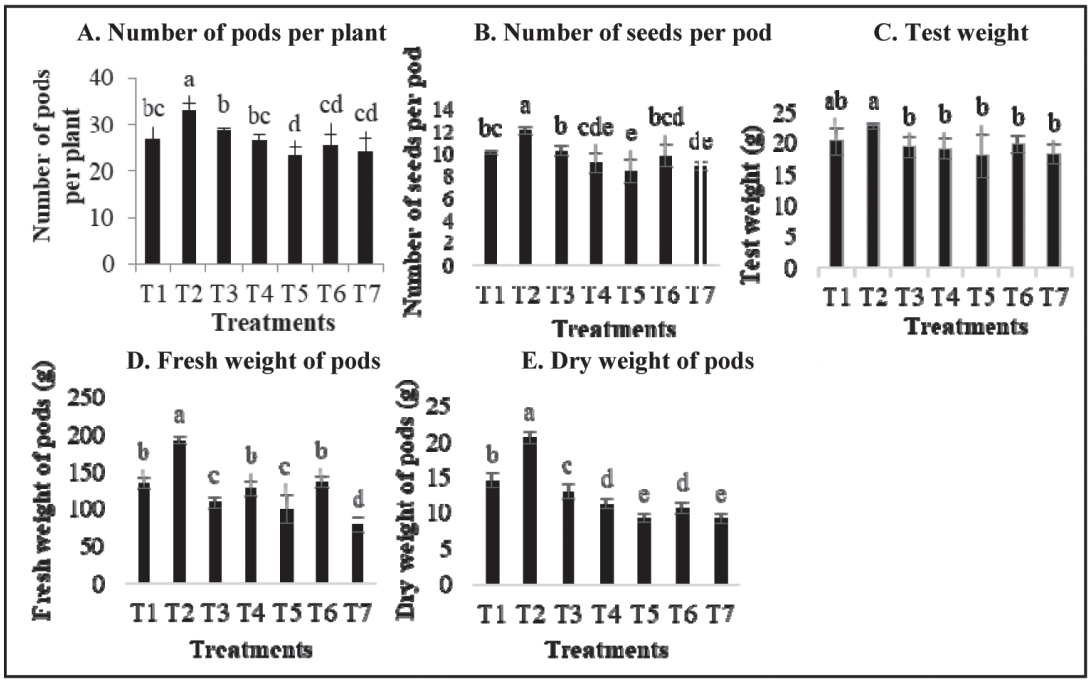
(Sreevidya et al., 2016). Previously it was reported that the culture filtrates of *Streptomyces olivaceoviridis* containing IAA stimulated growth and yield of wheat plants (Aldesuquy et al., 1998) and *Streptomyces* spp. from a tomato rhizosphere could produce IAA and improve tomato growth by increasing root dry weight (El-Tarabily, 2008). Htwe et al. (2019) observed that inoculation with *Streptomyces griseoflavus* enhanced agronomic traits, nodulation, and nitrogen fixation in soybean, cowpea, and mungbean. Based on these findings, it can be concluded that cowpea plants treated with the consortium containing DPS-7 and Cc-5 showed significantly increased root colonization efficiency, resulting in enhanced growth and yield.

*Identification of potential actinobacteria using 16S rRNA gene sequencing*

Among five promising PGPR actinobacterial consortial formulations tested under *inplanta* conditions, consortium2 consisting of DPS-7 and Cc-5 were subjected to molecular characterization by 16S rRNA gene sequencing. After identification, BLASTn homology analys is revealed that DPS-7 exhibited maximum homology with *Streptomyces* sp. and Cc-5 with *Streptomyces* sp. strain PAS3. Therefore, these were identified as*Streptomyces* sp. and Cc-5 with *Streptomyces* sp. and named as *Streptomyces* sp. strain DPS-7 and*Streptomyces*sp. strain Cc-5, respectively.

## Conclusion

This study highlights the potential of actinobacterial consortia as effective biofertilizers for promoting cowpea growth. The isolation, screening, and evaluation of actinobacterial isolates demonstrated significant plant growth-promoting activities, including solubilization of minerals, nitrogen fixation, production of phytohormones and siderophores. Among the evaluated consortia, the $T_2$ consortium, composed of *Streptomyces* sp. strain DPS-7 and *Streptomyces* sp. strain Cc-5, showed the most promising results in enhancing cowpea growth and yield. These findings suggest that actinobacterial consortia can be eco-friendly alternatives to chemical fertilizers, contributing to sustainable agricultural practices. Conducting field trials is crucial for formulating effective biofertilizers and plant growth-promoting strategies for use in commercial crop production systems. Future investigations may focus on isolating actinobacteria from less explored habitats, such as extreme environments, marine ecosystems, and specialized ecological niches, which hold significant promise for the discovery of novel compounds and innovative applications. Emphasis can be placed on characterizing the secondary metabolites produced by these




**$T_1$:** Consortium 1 (DPS-7 + Cc-5);
**$T_2$**: Consortium 2 (WA-27 + Cc-2);
**$T_3$**: Consortium 3 (Cc-5 + Cc-6);
**$T_4$**: Consortium 4 (WA-26 + DPS-5);
**$T_5$**: Consortium 5 (Cc-4 + DPS-7);
**$T_6$**: PGPR Mix 1 of KAU;
**$T_7$**: Control (Uninoculated)

*Figure 6.* Effect of actinobacterial consortia on yield parameters (A-E) in cowpea. Values were expressed as mean ± standard error. Different letters indicate significant differences at *P* d" .05 and treatments with same letters are not significantly different. A. Number of pods per plant, B. Number of seeds per pod, C. Test weight of seeds, D. Fresh weight of pods and E. Dry weight of pods.

microorganisms for their potential antimicrobial, antifungal, and biostimulant activities. Additionally, their capacity to alleviate abiotic stresses, including drought, salinity, and nutrient deficiencies, may be further investigated to enhance their application in stress-resilient agricultural systems.

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## References

Ahmad, F., Ahmad, A.I. and Khan M.S. (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microb. Res. 163: 173-181. doi: 10.1016/j.micres.2006.04.001.

Aldesuquy, H., Mansour, F. and Abo-Hamed, S. (1998) Effect of the culture filtrates of *Streptomyces* on growth and productivity of wheat plants. *Folia Microbiologica*. 43: 465-470.

Al-Hussini, H.S., Al-Rawahi, A.Y., Al-Marhoon, A.A., Al-Abri, S.A., Al-Mahmooli, I.H., Al-Sadi, A.M. and Velazhahan, R. (2019) Biological control of damping-off of tomato caused by *Pythium aphanidermatum* by using native antagonistic rhizobacteria isolated from Omani soil. *J. Plant Pathol.*101: 315-322. doi: 10.1007/s42161-018-0184-x

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

Archana, D.S. (2007) Studies on potassium solubilizing bacteria. M.Sc. (Ag) thesis, University of Agricultural Sciences, Department of Agricultural Microbiology. Dharwad.

Asiwe, J.A.N. (2006) Recent Progress in Cowpea Breeding at Agricultural Research Council (ARC)-Grain Crop Institute, Potchefstroom, South Africa. In I International Conference on Indigenous Vegetables and Legumes. *Prospectus for Fighting Poverty, Hunger and Malnutrition*752: 621-623. doi:10.17660/ActaHortic.2007.752.117

Backer, A. (2021) Plant growth promoting actinobacteria from rhizosphere soils of black pepper in Wayanad. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur 127p.

Benson, D.R. and Silvester, W.B. (1993) Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants. *Microbiol.* rev. 57(2): 293-319.doi:10.1128/mr. 57.2.293-319.1993

Bhatti, A.A., Haq, S. and Bhat, R.A. (2017) Actinomycetes benefaction role insoiland plant health. *Microb. Pathog.* 111: 458-467. doi:10.1016/j.micpath.2017.09.036

Bremner, J.M. (1960) Determination of nitrogen in soil by the Kjeldahl method. *J. Agric. Sci.* 55(1): 11-33.doi:10.1017/S0021859600021572

Burkholder, P.R., Sun,S.H., Ehrlich,J. and Anderson, L. (1954) Criteria of speciation in the genus *Streptomyces*. *Annu. New York Acad. Sci.*60(1):102-123.

Cappuccino, J.C. and Sherman, N. (1992) In: Microbiology: A Laboratory Manual, New York 125-179.

Cheng, Y.Q., Yang, R.J., Lyu, M., Wang, S.W., Liu, X.C., Wen, Y., Song, Y., Li, J. and Chen, Z.(2018) IdeR, a DtxR family iron response regulator, controls iron homeostasis, morphological differentiation, secondary metabolism, and the oxidative stress response in *Streptomyces avermitilis*. *Appl. Environ. Microbiol.* 84: 01503-18. doi:10.1128/AEM.01503-18

Cowan, S.T. (1974) Cowan and Steel's Manual for the identification of medical bacteria, 2nd ed., Cambridge.

Dochhil, H., Dkhar, M.S. and Barman, D. (2013) Seed germination enhancing activity of endophytic *Streptomyces* isolated from indigenous ethnomedicinal plant *Centella asiatica*. I*nt. J. Pharma. Bio. Sci* 4(1): 256-262.

Drummond, D.A. and Wilke, C.O. (2010) Signatures of protein biophysics in coding sequence evolution. *Curr. Opin. Struct. Biol.* 20(3): 385-389.doi:10.1016/j.sbi.2010.03.004

El-Tarabily, K.A. (2008) Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing Streptomycete actinomycetes. *Plant Soil* 308: 161-174. doi:10.1007/s11104-008-9616-2

El-Tarabily, K.A., AlKhajeh, A.S., Ayyash, M.M., Alnuaimi, L.H., Sham, A., ElBaghdady, K.Z., Tariq, S. and AbuQamar, S.F. (2019) Growth promotion of *Salicornia bigelovii* by *Micromonospora chalcea* UAE1, an endophytic 1-aminocyclopropane-1-carboxylic acid deaminase-producing actinobacterial isolate. *Front. Microbiol.*10: 1694.

El-Tarabily, K.A., Sham, A., Elbadawi, A.A., Hassan, A.H., Alhosani, B.K., El- Esawi, M.A., AlKhajeh, A.S. and AbuQamar, S.F. 2021. A consortium of rhizosphere-competent actinobacteria exhibiting multiple plant growth- promoting traits improves the growth of *Avicennia marina* in the United Arab Emirates. *Front. Marine Sci.* 8:715123.

Etesami, H., Emami, S. and Alikhani, H.A. (2017) Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects A review. *J. Soil Sci. Plant Nutr.* 17(4): 897-911. doi:10.4067/S0718-95162017000400005

Franche, C., Lindström, K. and Elmerich, C. (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant Soil.*321: 35-59.

Franco-Correa, M. and Chavarro-Anzola, V. (2016) Actinobacteria as plant growth promoting rhizobacteria. Actinobacteria-basis and biotechnological application: 249-270.

Glick, B.R. (2012) Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica.* 2; 1-15. doi:10.6064/2012/963401

Gopalakrishnan, S., Humayun, P., Vadlamudi, S., Vijayabharathi, R., Bhimineni, R.K. and Rupela, O. (2012) Plant growth-promoting traits of Streptomyces with biocontrol potential isolated from herbal vermicompost. *Biocontrol Sci. Technol.* 22(10): 1199-1210. doi:10.1080/09583157.2012.719151

Gopinath, P.P., Parsad, R., Joseph, B. and Adarsh, V.S. (2021) GrapesAgri1: collection of shiny apps for data analysis in agriculture. *J. Open Source Softw.* 6(63): 3437.doi:10.21105/joss.03437

Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I. (2010) Soil beneficial bacteria and their role in plant growth

promotion: a review. *Ann. Microbiol.*60: 579-598.

Htwe, A.Z., Moh, S.M., Soe, K.M., Moe, K. and Yamakawa, T. (2019) Effects of biofertilizer produced from *Bradyrhizobium* and *Streptomyces griseoflavus* on plant growth, nodulation, nitrogen fixation, nutrient uptake, and seed yield of mung bean, cowpea, and soybean. *Agronomy* 9(2): 77.doi:10.3390/agronomy9020077

Hucker, G.J. and Conn, H.J. (1923) Methods of Gram staining.

Jackson, M.L. (1973) Soil Chemical Analysis. (Indian reprint, 1976), Prentice Hall of India, New Delhi 478.

Johnson, L.F. and Curl, E.A. (1972)Methods for research on the ecology of soil borne plant pathogens, Burgess, Minneapolis. *J. Biotechnol.* 7(8): 967-972.

Khamna, S., Yokota, A., Peberdy, J.F. and Lumyong, S. (2010) Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *Eur. Asian J. Biol. Sci.* 4: 23-32.10.5053/ejobios.2010.4.0.4

Khoshru, B., Mitra, D., Khoshmanzar, E., Myo, E.M., Uniyal, N., Mahakur, B., Mohapatra, P.K., Panneerselvam, P., Boutaj, H., Alizadeh, M. and Cely, M.V.(2020) Current scenario and future prospects of plant growth-promoting rhizobacteria: an economic valuable resource for the agriculture revival under stressful conditions. *J. Plant Nutr.*43(20): 3062-3092. doi:10.1080/01904167.2020.1799004

Law, J.W.F., Tan, L.T.H., Letchumanan. V., Hong, K.W., Ser, H.L., Goh, B.H., Ab Mutalib, N.S., Chan, K.G. and Lee, L.H. (2023) *Streptomyces griseiviridis* sp. nov., a novel "modern actinobacteria" isolated from Malaysia mangrove soil. *Prog. Microbes Mol. Biol.* 6(1): 0000270.

Lewin, G.R., Carlos, C., Chevrette, M.G., Horn, H.A., McDonald, B.R., Stankey, R.J., Fox, B.G. and Currie, C.R. (2016) Evolution and ecology of Actinobacteria and their bioenergy applications. *Annu. Rev. Microbiol.*70(1): 235-254.

Li, Q., Chen, X., Jiang, Y. and Jiang, C. (2016) Morphological identification of actinobacteria. Intech open.

Lorck, H. (1948) Production of hydrocyanic acid by bacteria. *Physiol. Plant.* 1948: 1(2).10.1111/j.1399-3054.1948.tb 07118.x

Mitra, D., Mondal, R., Khoshru, B., Senapati, A., Radha, T.K., Mahakur, B., Uniyal, N., Myo, E.M., Boutaj, H., Sierra, B.E. and Panneerselvam, P.(2022) Actinobacteria-enhanced plant growth, nutrient acquisition, and crop protection: Advances in soil, plant, and microbial multifactorial interactions. *Pedosphere* 32(1): 149-170.doi:10.1016/S1002-0160(21)60042-5

Nalini, B.S., Muthuraju, R., Vendan, T., Brahmaprakash, G.P., YA, N.R., Nagaraju, N. and Anil, V.S. (2020) Isolation of plant growth promoting actinobacteria from the rhizosphere of finger millet and cowpea. *J. Pharmacogn. Phytochem.*9(6): 1107. doi:10.22271/phyto.2020.v9.i6p.13097

Nguyen, C., Yan, W., Le Tacon, F. and Lapeyrie, F. (1992) Genetic variability of phosphate solubilizing activity by monokaryotic and dikaryotic mycelia of tht ectomycorrhizal fungus *Laccaria bicolor* (Maire) PD Orton. *Plant Soil* 143(2): 193-199. doi:10.1007/BF00007873

Olsen, S.R., Kemper, W.D. and Jackson, R.D. (1962) Phosphate diffusion to plant roots. *Soil Sci. Soc. Am. J.* 26(3): 222-227. doi:10.2136/sssaj1962.03615995002600030011x

Passari, A.K., Mishra, V.K., Gupta, V.K., Yadav, M.K., Saikia, R. and Singh, B.P. (2015)*In vitro* and *in vivo* plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants.*PLoS ONE.* 1-18. doi:10.1371/journal.pone.0139468

Patel, K.B. and Thakker, J.N. (2020) Deliberating plant growth promoting and mineral-weathering proficiency of *Streptomycesnanhaiensis* strain YM4 for nutritional benefit of millet crop (*Pennisetumglaucum*). *J Microbiol. Biotechnol. Food Sci.* 9(4):7 21-726.10.15414/jmbfs. 2020.9.4.721-726

Praveen, K.G., Leo, D.A.E., Suseelendra, D. and Mir, H.A.S. (2013) Prospective zinc solubilizing bacteria for enhanced nutrient uptake and growth promotion in maize (*Zea mays* L.). *Int. J. Microbiol. App. Sci.* 6(11): 3058-3065.

Rosenberg, E., Sharon, G. and Zilber-Rosenberg, I. (2009) The hologenome theory of evolution contains Lamarckian aspects within a Darwinian framework. *Environ. Microbiol.* 11(12): 2959-2962. doi:10.1111/j.1462-2920.2009.01995.x

Sambrook, J. (1989) Molecular cloning: a laboratory manual. Cold Spring Habor Laboratory.

Saravanan, V.S., Subramoniam, S.R. and Raj, S.A. (2004) Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Braz. J. Microbiol*.35: 121-125. doi:10.1590/S1517-83822004000100020

Schwyn, B. and Neilands, J. (1987) Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*160(1): 47-56. doi:10.1016/0003-2697(87)90612-9

Solans, M., Vobis, G., Cassán, F., Luna, V. and Wall, L.G. (2011) Production of phytohormones by root-associated saprophytic actinomycetes isolated from the actinorhizal plant *Ochetophilatrinervis*. *World J. Microbiol. Biotechnol*.27: 2195-2202. doi:10.1007/s11274-011-0685-7

Sreevidya, M., Gopalakrishnan, S., Kudapa, H. and Varshney, R.K. (2016) Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. *Braz. J. Microbiol.*47: 85-95. doi:10.1016/j.bjm.2015.11.030.

Srinivas, V., Sravani, A., Pratyusha, A. and Gopalakrishnan, S. (2021) Amazing Plant Growth-Promoting Actinobacteria from Herbal Vermicompost. *Andhra Pradesh J. Agril. Sci.*7(2): 89-98. http://oar.icrisat.org/id/eprint/11951

Sugumaran, P. and Janarthanam, B. (2007) Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World J. Agric. Sci.* 3(3): 350-355. http://www.idosi.org/wjas/wjas3(3)/14.pdf

Suksaaid, P., Pathom-aree, W. and Duangmal, K. (2017) Diversity and plant growth promoting activities of actinomycetes from mangroves. Chiang Mai. J. Sci. 44(4): 1210-1223. http://epg.science.cmu.ac.th/ejournal/.

Taylor, W.I. and Achanzar, D. (1972) Catalase test as an aid to the identification of Enterobacteriaceae. *Appl. Microbiol.*24(1): 58-61.doi:10.1128/am.24.1.58-61.1972

Thampi, A. and Bhai, R.S. (2017) Rhizosphere actinobacteria for combating *Phytophthora capsica* and *Sclerotium rolfsii*, the major soil borne pathogens of black pepper. *Biol. Cont.* 109: 1-13. doi:10.1016/j.biocontrol.2017.03.006.

Thenappan, D.P., Pandey, R., Hada, A., Jaiswal, D.K., Chinnusamy, V., Bhattacharya, R. and Annapurna, K. 2024. Physiological Basis of Plant Growth Promotion in Rice by Rhizosphere and Endosphere Associated *Streptomyces* Isolates from India. *Rice*. 17(1): 60.

Tokala, R.K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, M.H., Deobald, L.A., Bailey, J.F. and Morra, M.J. (2002) Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). *Appl. Environ. Microbiol.* 68(5): 2161-2171.

Uesugi, J.H.E., dos Santos Caldas, D., Coelho, B.B.F., Prazes, M.C.C., Omura, L.Y.E., Pismel, J.A.R. and Bezerra, N.V. (2024) Morphological diversity of actinobacteria isolated from oil palm compost (*Elaeis guineensis*). *Braz. J. Microbiol.* 55(1): 455-469.

Vo, Q.T., Ballard, R.A., Barnett, S.J. and Franco, C.M. (2021) Isolation and characterisation of endophytic actinobacteria and their effect on the growth and nodulation of chickpea (*Cicer arietinum*). *Plant Soil.* 466: 357-371.doi:10.1007/s11104-021-05008-6

Vyawahare, S.S., Kamble, K.D., Waghmare, V.D. and Kamble, L.H. (2013) Characterization of actinomycetes for some industrially important enzymes. *Trends Biotech. Res.* 2(2): 2320-0421.

Wang,Z., Solanki, M.K., Yu, Z.X., Yang, L.T., An, Q.L., Dong, D.F. and Li,Y.R. (2019) Draft genome analysis offers insights into the mechanism by which *Streptomyces chartreusis* WZS021 increasesd rought tolerance in sugarcane. *Front. Microbiol*.9: 3262.

Woliñska, A., Górniak, D., Zielenkiewicz, U., KuŸniar, A., Izak, D., Banach, A. and B³aszczyk, M.(2019) Actinobacteria structure in autogenic, hydrogenic and lithogenic cultivated and non-cultivated soils: a culture-independent approach. *Agronomy* (10): 598. doi:10.3390/agronomy 9100598

Yadav, A.N., Verma, P., Kumar, S., Kumar, V., Kumar, M., Sugitha, T.C., Singh, B.P., Saxena, A.K. and Dhaliwal, H.S.(2018) Actinobacteria from rhizosphere: molecular diversity, distributions, and potential biotechnological applications. In New and future developments in microbial biotechnology and bioengineering. 13-41. doi:10.1016/B978-0-444-63994-3.00002-3

Yasari, E., Mozafari, S., Shafiee, E. and Foroutan, A. (2009) Evaluation of sink-source relationship of soybean cultivars at different dates of sowing. *Res. J. Agric. Biol. Sci.*5(5): 786-793