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MICROBIOLOGY

The Distinctive Endophytic Bacterial Isolates Obtained from Coffee (*Coffea arabica* L.) RCRS, Thandigudi

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ABSTRACT

The study aimed to identify and determine the endophytic bacteria of coffee leaves (*Coffea arabica* L.) having multifunctional plant growth-promoting traits. Coffee leaves were obtained from the plants grown at the Regional Coffee Research Station of Lower Pulney hills, Thandigudi. Examination of seven distinct endophytic bacterial isolates was characterized morphologically and biochemically. Endophytic bacterial isolates have shown various levels of resistance in recent research. Plant growth-promoting traits include nitrogen fixation, phosphate solubilization, and potassium mobilization. Also, the endophytic microbiota were the potential plant symbionts for conferring biotic and abiotic stress tolerance. Based on the results, the two efficient isolates had undergone 16S rRNA sequencing, and the results showed that the SE4 was *Chryseobacterium indologeness*, IMP2 was *Pseudomonas putida*. It has been found that both possessed a high range of antagonistic activity in terms of percent growth inhibition (GI%) against *Pythium* spp. *Macrophomina* spp. and *Fusarium* spp. And the developed module could be recommended as an effective biocontrol agent against disease-causing pathogens and an excellent plant growth regulator for sustainable production of various crops, without hamperingsoil health and fertility.

HIGHLIGHTS

- Plant growth promoting potential of endophytic bacterial isolates was assessed in coffee leaves.
- The investigated isolates showed an effective antagonism against a wide range of phytopathogens.

Keywords: Coffee, Endophytic bacteria, Plant growth promotion, Antagonistic activity

Coffee (*Coffee arabica* L.) is considered the most preferred beverage in the world, which belongs to the family Rubiaceae. Its consumption is primarily around tropical and subtropical regions. As the most essential beverage, coffee has a significant economic impact. There are about 120 species in the coffee genera. The only two species cultivated for commercial purposes are *Coffea arabica* L. and *Coffea canephora* L. *Coffea arabica* L. cultivation ranges from 60 to 80 percent, whereas *Coffea canephora* L. was approximately around 20 to 40 percent (Davis *et al*. 2019).

Hundreds of physiologically active phytochemicals are present in coffee. During the roasting of coffee, polyphenols like chlorogenic acid and lignans, the alkaloid trigonelline, and melanoidins are generated. Coffee caffeine contains moderate levels of magnesium (Mg), potassium (K), and vitamin B_3 (Niacin) (Dam *et al*. 2020). The risk of several chronic diseases has been reduced due to the consumption of 3 – 5 cups of coffee daily. Adults are recommended to consume 400 g of caffeine daily, while pregnant and lactating women could consume 200 g (European Food Safety Authority (EFSA, 2021). Brazil, Vietnam, Colombia, Indonesia,

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Ethiopia, India, and Honduras were the major coffee-producing countries. It generates more than 120 million employment and is a crucial source of cash for more than 40 tropical nations. In India, the total production of coffee was 334000 million tonnes, and the production of coffee in Tamil Nadu was 18240 million tonnes (2020-2021) (ICO Statistics on coffee, 2022).

Tamil Nadu stands in second place in coffee production, with 17,875 metric tonnes produced in the previous fiscal year. Tamil Nadu is a significant coffee-growing state, generating more than 5 percent of the country's total coffee. Arabica Coffee accounts for most of the state's overall coffee output, with 13,150 metric tonnes produced in the previous fiscal year. The state also produces Robusta Coffee, with 4,725 metric tonnes produced in the previous year. Tamil Nadu's primary coffee-growing regions include Pulneys, Nilgris, Salem, and Coimbatore. Pulneys includes lower and higher hills production was more than 7,600 Metric tonnes of coffee, making it the state's highest coffee-producing region. At lower pulney hills, one of the most beautiful villages named, Thandigudi was located at a latitude of 10.31 and a longitude of 77.64. The present study has been conducted at the Thandigudi village's Regional Coffee Research Station.

Improving the sustainability of the agricultural production system is an aspiration nowadays. Developing a sustainable agricultural ecosystem depends on proper land usage, water and soil management, and various socioeconomic aspects. Endophytes and plant growth-promoting microorganisms are free-living microbes that colonize plant roots and other aerial plant tissues and promote crop growth. PGPs increase plant growth by enhancing their metabolism, stimulating the plant's enzymatic activity, "assisting" other beneficial microbes and their capacity to enhance the growth and sustainability of the crop. Bacteria are one of the most prominent microbes in the rhizosphere. They impact plant physiology and colonization (Barriuso *et al*. 2008). The Rice phyllosphere is the habitat of these bacteria, and their physiological response to the surrounding atmosphere has gotten less attention. A wide range of bacterial isolates from the rice phyllosphere have also been reported (Elbeltagy *et al*. 2000; Mano *et al*. 2008).

Phyllospheric bacteria have the credentials of plant growth enhancement, which is involved directly by producing enzyme 1-aminocyclopropane 1-carboxylate. This enzyme reduces plant ethylene levels and is indirectly implicated in pathogen infection prevention by producing anti-bacterial compounds that provide the plant systemic resistance. These qualities are essential in agriculture to enhance soil fertility and crop productivity while reducing synthetic fertilizers' negative adverse environmental effects.

PGPR was first reported as a type of bacterium having plant growth-promoting characteristics that were resistant to plant diseases. Plant growth-promoting effects include nitrogen fixation, solubilisation solubilization of phosphorous, and organic matter mineralisation mineralization (Melnick *et al.* 2008). The strains generate phosphatase and Indole acetic acid in vitro. The most investigated genera include *Alcaligenes, Pseudomonas, Chryseobacterium, Azospirillum, Bacillus, Klebsiella, Azotobacter, Enterobacter, Gluconacetobacter, Burkholderia, Arthrobacter, Rhizobium, Bradyrhizobium,* and *Serratia*.

The most frequent coffee leaf disease is a severe constraint to coffee output in the pulney hills, causing production losses of up to 35-40% on average and up to 60% in extreme circumstances (Gouveia *et al.* 2005). *Hemileia vastatrix,* the typical coffee leaf rust disease causing phytopathogen, creates a nasty infection that lowers the quality and quantity of coffee berries. Endophytic bacteria isolated from coffee leaves can act as plant growth stimulants and biocontrol agents. Endophytic bacterial strains are more successful in controlling leaf rust and promoting plant development than endophytic fungi isolates.

MATERIALS AND METHODS

Endophytic bacterial isolates of coffee

For the current research, the coffee leaves were collected from Coffee plantations of RCRS. Leaves selected for the subsequent studies were clipped at early stages (greenish to bronze) colored.

Coffee Leaf Extraction method

The leaf extraction method was carried out under aseptic conditions. The leaves were nipped off using

sterile forceps and needles. One gram of fresh leaf sample was weighed. Endophytic bacteria that have been isolated from the coffee plants were taken. Then surface sterilization with 2 %(v/v) NaOCl, for 1 minute and 30% (v/v) H_2O_2 for 30 seconds followed by 70% (v/v) ethyl alcohol for 1 minute and 30% (v/v) H_2O_2 for 30 seconds simultaneously. Then washed thoroughly with sterilized distilled water. The leaf sample was crushed with a sterile pistil and mortar until it became a smooth paste. The cultures were isolated by pour plate technique and incubated at 27±2°C for 24 hours. Purifying isolates was done after the incubation period was over and maintained in LB slants at 4°C for further studies.

Imprintation method

Agar media was prepared for the dilution plating procedure. Imprints of leaves were made on agar media. 0.5% sodium hypochlorite and 70% ethyl alcohol were used to surface the coffee leaves, each for two minutes. Finally, sterile distilled water was used for rinsing and wiping off. The samples were cut into 2×3 sizes and placed in a (5 cm) diameter plate containing Yeast malt extract agar. Different leaves were utilised to imprint the adaxial and abaxial surfaces to minimize disruptions in surface community dispersion. The plates were incubated at 24°C at 27±2°C for 2-5 days.

Morphological characterisation

Bacterial endophytes were classified based on phenotypic characteristics such as colony color, margin, shape, texture, gram reaction, elevation, and spore-forming abilities (Steinbach and Shetty, 2001).

Gram staining (Hucker and conn, 1923)

Hucker's modified technique was used to stain the inoculants using Gram staining.

Endospore staining (Hussey and Zayaitz, 2007)

The standard method for the endospore staining technique is the Schaeffer-Fulton method (or a modification).

Motility test (Jain, 2020)

Fresh culture of bacteria was taken. Then bacterial motility was determined using Microscopy. The microscopic technique commonly used is the 'hanging drop method' of Jain.

Biochemical characterisation

Biochemical tests did identification of bacteria for tentative characterization.

Catalase test (Aneja, 2006)

A loop containing a 24-hour-old culture of an endophytic bacterial isolate grown on nutritional agar slants was dipped into a glass tube containing 0.5 ml distilled water and mixed thoroughly; take one drop of culture and place it in the glass slide. Add a few drops of 3% hydrogen peroxide. To detect the presence of enthusiasm.

Oxidative fermentation test (Aneja, 2006)

Fermentation media was prepared. The media was autoclaved at 15lb pressure for 15 minutes. Each specified fermentation tube of media was inoculated with the cultures and labeled. A layer of oil was added on top of the media in a test tube. Incubated at 35°C for 24-48 hours and observed for color change.

IAA test (Aneja, 2006)

The test cultures were inoculated into tubes containing 1% tryptone broth and cultured at 35°C for 48 hours. After the incubation time, the tubes were filled with 1 ml of Kovac's reagent, and the observations were recorded.

Methyl Red test (Aneja, 2006)

The endophytic bacterial isolates were inoculated with Methyl Red test broth made in a set of test tubes, and the incubation temperature was at 30°C for 48 hours. Few drops of methyl red alcoholic solution were added to the set of test tubes. The appearance of a distinct red hue predicted positive MR test results.

Voges-proskauer test (Aneja, 2006)

The endophytic bacterial isolates were inoculated in the set of tubes. A naphthol solution (5% solution in 70% ethyl alcohol) was added. Then shook for 15 minutes. The emergence of a red hue suggested a favorable response of acetyl methyl carbinol synthesis. This showed a positive VP test result.

Citrate utilization test (Aneja, 2006)

Inoculations of endophytic bacterial isolates into Simmons citrate agar medium were done. Culturing is made for 48 hours at 37°C. A change in pH causes the medium to discolor from green to blue, indicating a favorable response.

Starch hydrolysis test (Aneja, 2006)

Make a single line streak across the plate with the unknown microorganism. Incubate at either 25 or 37°C. Flood the plate with iodine after incubation and development.

Urease test (Aneja, 2006)

The endophytic bacterial isolates were inoculated to urea agar plates and the incubation period was up to 24 to 48 hours. The medium's hue shifts from blue to red.

Hydrogen sulphide production (Aneja, 2006)

The production of hydrogen sulphide was studied using a sulphide indole motility (SIM) medium. SIM agar stabs were made, and endophytic bacterial isolates were inoculated into them. Stabs were inoculated, and the incubation period was at 30°C for 48 hours. The results were observed and recorded.

TSI test (Aneja, 2006)

Test cultures were streaked above the agar slants and incubated for 24 hours at 37 °C with a loose top to allow for air ambiance. The color indication from orange to deep reddish was observed.

Casein hydrolysis (Aneja, 2006)

Endophytic bacterial cultures were inoculated in plates consisting of milk agar medium. The incubation period was at 37°C for 24-48 h. The results were observed.

Carbon utilization of different bacterial endophytes (Kennedy, 1994)

The carbon utilization ability of seven different endophytic plant growth promoting bacterial isolates was examined. One per cent concentration of glucose, sucrose, fructose, maltose, and lactose was added. The cultures were inoculated into the test tubes with the carbohydrate fermentation medium. Incubate the cultures at room temperature for 2 to 3 days. Inspect the tubes for gas and acid production.

Molecular analysis

Characterizing selected endophytic bacterial cultures was based on the sequencing software v1 for 16S rRNA gene and phylogenesis investigation. The sequence alignment and modification were done by MEGA 7.0. NCBI's BLAST program's similarity index was used for the sequence amplification and validation. The species used in this research were assigned to the following species based on their higher percentage resemblance to the species of the same genera.

Antagonistic nature of endophytic bacteria (Santos *et al.* 2008)

The 24-48 h old selected endophytic bacterial isolates were taken for the antagonistic analysis against three different phytopathogens *viz., Macrophomina* spp. *Pythium* spp. and *Fusarium* spp. by the confrontation assay method. The following three phytopathogen discs with 0.5 diameters were positioned on one side of the PDA media (Hi media) plates, and the test culture was streaked as a straight line on the other. The incubation temperature was maintained at 28°C for 5-7 days to observe the results. The antagonistic activity of the test cultures against phytopathogens was measured and converted as the percentage by the mycelial growth inhibition formula,

$$\text{Mycelial Growth Inhibition (\%)} = I_0 - I_1/I_0 \times 100$$

Where, I_0 = growth of the phytopathogen in plates without isolates,

I_1 = growth of the phytopathogen in plates with isolates (Dual culture technique).

RESULTS AND DISCUSSION

Isolation and molecular characterization of endophytic bacterial isolates

The distinct endophytic bacterial isolates were obtained from the coffee leaves located at Regional Research Station, Thandigudi. The isolates were designated as SE1, SE2, SE3, SE4, and SE5 were

isolated from coffee leaf extracts, and IMP1, and IMP2 from the leaf implantation method were arranged sequentially (Fig. 1).

Seven different Plant growth promoting isolates were studied. The morphological and biochemical testing was conducted to identify the species of the genus. The morphological characteristics of the bacteria were obtained using Bergey's handbook of systematic bacteriology. Endophytic bacterial isolates from coffee leaves were tentatively identified as *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Chryseobacterium*by biochemical analysis, and the details are included in (Tables 1 and 2).

Carbon utilization of endophytic bacterial isolates

The PGP endophytic bacterial isolates utilized various carbon sources, including glucose, fructose, maltose, lactose, and sucrose. According to studies, most endophytic bacterial isolates utilized carbon sources and have an enormous amount of acid and gas production (Table 3).

Molecular analysis of effective endophytic isolates

The effective isolates of endophytic bacteria were closely related to *Chryseobacterium* (SE2) and *Pseudomonas* (IMP2) genera. The MEGA 7.0 phylogenetic analysis was carried out, and the closely related sequences were aligned, and a neighbor joining tree was generated (Figs. 3 and 4). Their 16S rRNA sequence reports were submitted to the NCBI gene bank under the accession codes ON258638 and ON103518.

Antagonistic nature of effective endophytes against phytopathogens

Antagonistic activity of selected endophytic bacterial isolates such as *Chryseobacterium indologenss* and *Pseudomonas putida* has shown excellent antagonistic activity against pathogenic fungi *viz*., *Macrophomina* spp. *Pythium* spp. and *Fusarium* spp. ranged between 48-72%, 40-60%, and 35-50% approximately to the activity of mycelial growth inhibition as given

Fig. 1: Isolation of Endophytic bacterial isolates from Coffee leaves (*Coffee arabica* L.) by Leaf extraction method

Fig. 2: Isolation of Endophytic bacterial isolates from Coffee (*Coffea arabica* L.) by Leaf Imprintation method

Table 1: Morphological characterization of isolated bacterial strains from coffee (*Coffea arabica* L.)

Isolate code	Margin	Colony colour	Shape	Texture	Gram reaction	Elevation	Mobility	Spore forming	Tentative Identification
SE1	Entire	White dense	Rod	Slightly curved	Negative	Convex	Nonmotile	Negative	*Azospirillum lipoferum*
SE2	Irregular	Grey white	Rod	Rough	Positive	Flat	Motile	Central spore	*Bacillus subtilis*
SE3	Unbonate	White	Rod	Dull	Positive	Convex	Motile	Positive	*Bacillus megaterium*
SE4	Regular margin	Yellow	Rod	Circular	Negative	Low convex	Nonmotile	Negative	*Chryseobacterium indologenss*
SE5	Wavy	Diffusible green	Oval and medium	Mucoid	Negative	Unbonate	Nonmotile	Negative	*Pseudomonas* spp.
IMP1	Irregular	Grey white	Rod	Dull	Positive	Flat	Motile	Positive	*Bacillus* spp.
IMP2	Smooth	Fluorescent	Rod	Mucoid	Negative	Unbonate	Nonmotile	Central spore	*Pseudomonas putida*

Table 2: Biochemical characterization of isolated bacterial strain from Coffee (*Coffea arabica* L.)

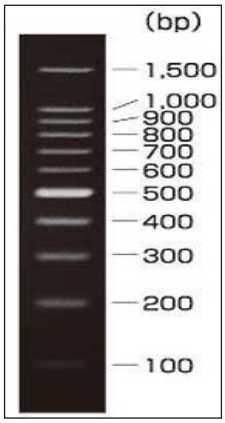
Isolate code	Catalase test	OF test	Urease test	IMvic test				Hydrolysis of Starch	Hydrogen sulphide Production test	TSI test	Hydrolysis of Casein	Tentative identification
				IAA	MR test	VP test	Utilization of Citrate					
SE1	+++	+	+++	-	-	+	+	+++	-	-	-	*Azospirillum lipoferum*
SE2	+++	+	-	-	-	+	+	-	+	-	+	*Bacillus subtilis*
SE3	+++	+	-	-	-	-	+	+	+	-	-	*Bacillus megaterium*
SE4	+++	+	+/-	+	-	-	-	+	-	+	+	*Chryseobacterium indologenss*
SE5	+++	+	+	-	-	+	-	-	-	-	+	*Pseudomonas* spp.
IMP1	+++	+	-	-	-	+	+	-	+	-	+	*Bacillus* spp.
IMP2	+++	+	-		-	-	-	-	-	-	-	*Pseudomonas putida*

Table 3: Carbon utilization test of PGP endophytic bacterial isolates obtained from coffee (*Coffea arabica* L.)

Isolate code	Catalase test	OF test	Urease test	IMvic test				Hydrolysis of Starch	Hydrogen sulphide Production test	TSI test	Hydrolysis of Casein	Tentative identification
				IAA	MR test	VP test	Utilization of Citrate					
SE1	+++	+	+++	-	-	+	+	+++	-	-	-	*Azospirillum lipoferum*
SE2	+++	+	-	-	-	+	+	-	+	-	+	*Bacillus subtilis*
SE3	+++	+	-	-	-	-	+	+	+	-	-	*Bacillus megaterium*
SE4	+++	+	+/-	+	-	-	-	+	-	+	+	*Chryseobacterium indologenss*
SE5	+++	+	+	-	-	+	-	-	-	-	+	*Pseudomonas* spp.
IMP1	+++	+	-	-	-	+	+	-	+	-	+	*Bacillus* spp.
IMP2	+++	+	-		-	-	-	-	-	-	-	*Pseudomonas putida*

in Fig. 5. Our present study was experimented with and resulted based on the following research.

Similarly, Assumpaco *et al*. (2009) isolated and characterized various plant growth-enhancing endophytic isolates from soybean seeds, and the findings demonstrated that every isolate that produced IAA and particularly *Pseudomonas* strains, possesses remarkable antagonistic activity.



Ladder specification

Chryseobacterium indologenss

CTGGCTCAGGATGAACGCTAGCGGGAGGCCTAACACATGCAAGCCGAGCGGTAGAGATTCTTCGGAA
TCTTGAGAGCGGCGTACGGGTGCGGAACACGTGTGCAACCTGCCTTTATCTGGGGGATAGCCTTTCGA
AAGGAAGATTAATACCCCATAATATATTGAATGGCATCATTTGATATTGAAAACTCCGGTGGATAGAG
ATGGGCACGCGCAAGATTAGATAGTTGGTGAGGTAACGGCTCACCAAGTCAACGATCTTTAGGGGGCC
TGAGAGGGTGATCCCCCACACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGG
AATATTGGACAATGGGTGAGAGCCTGATCCAGCCATCCCGCGTGAAGGACGACGGCCCTATGGGTTGT
AAACTTCTTTTGTATAGGGATAAACCTAGATACGTGTATCTAGCTGAAGGTACTATACGAATAAGCAC
CGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATCCGGATTTATTGGGTTT
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ATACTGCAAGTCTTGAGTGTTGTTGAAGTAGCTGGAATAAGTAGTGTAGCGGTGAAATGCATAGATAT
TACTTAGAACACCAATTGCGAAGGCAGGTTACTAAGCAACAACTGACGCTGATGGACGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGCTAACTCGTTTTTGGGATGAA
AATTTCAGAGACTAAGCGAAAGTGATAAGTTAGCCACCTGGGGAGTACGAACGCAAGTTTGAAACTC
AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGATTATGTGGTTTAATTCGATGATACGCGAGGAAC
CTTACCAAGGCTTAAATGGGAAATGACAGGTTTAGAAATAGACTTTTCTTCGGACATTTTTCAAGGTGC
TGCATGGTTGTCGTCAGCTCGTGCCGTGAGGTGTTAGGTTAAGTCCTGCAACGAGCGCAACCCCTGTC
ACTAGTTGCCATCATTAAGTTGGGGACTCTAGTGAGACTGCCTACGCAAGTAGAGAGGAAGGTGGGG
ATGACGTCAAATCATCACGGCCCTTACGCCTTGGGCCACACACGTAATACAATGGCCGGTACAGAGGG
CAGCTACACTGCGAAGTGATGCAAATCTCGAAAGCCGGTCTCAGTTCGGATTGGAGTCTGCAACTCGA
CTCTATGAAGCTGGAATCGCTAGTAATCGCGCATCAGCCATGGCGCGGTGAATACGTTCCCGGGCCTT
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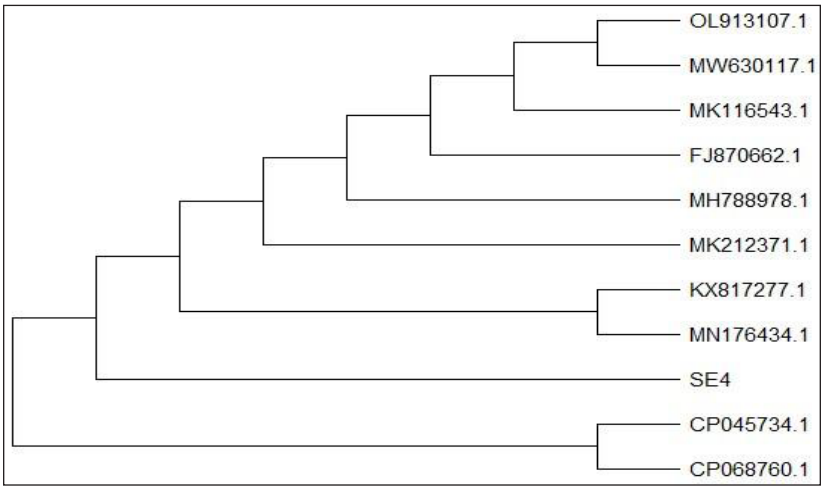
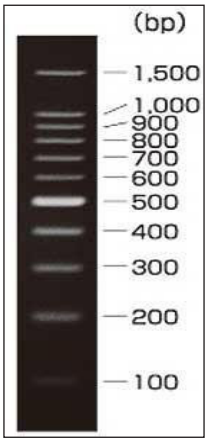
TAGGGTAAAACAGGTAACTAGGGC TAAGTCGTAA



Phylogenetic Tree of *Chryseobacterium indologenss*

Fig. 3: Molecular characterization of *Chryseobacterium indologeness* (SE4)



Ladder specification

Pseudomonas putida

TAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAC
GGGAGCTTGCTCCTTGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGG
ACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCC
TTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGAT
CCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGG
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ACCGACAGAATAAGCCCCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTA
ATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCA
ACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACAGTAGAGGGTGGTGGAATTTCCTGTGTAGC
GGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACAC
TGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTC
AACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGT
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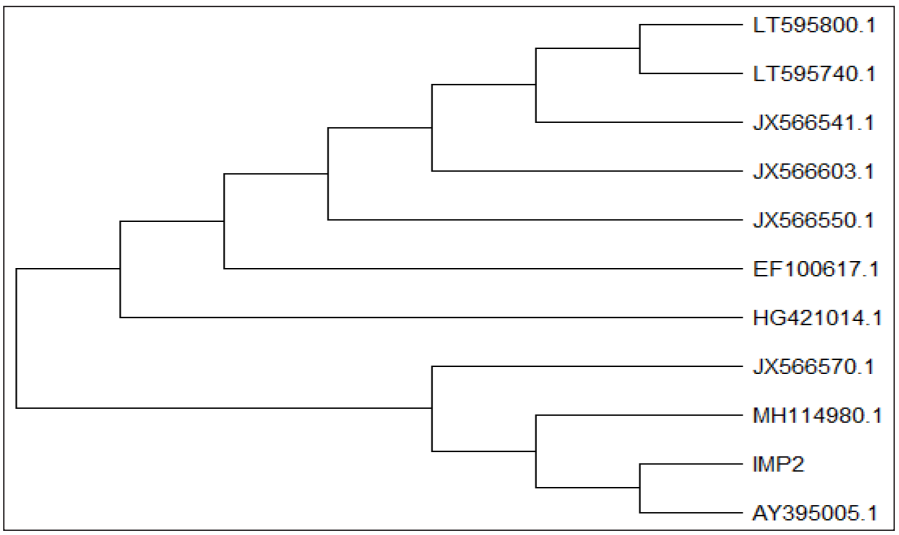

Phylogenetic Tree of *Pseudomonas putida*

Fig. 4: Molecular characterization of *Pseudomonas putida* (IMP2)

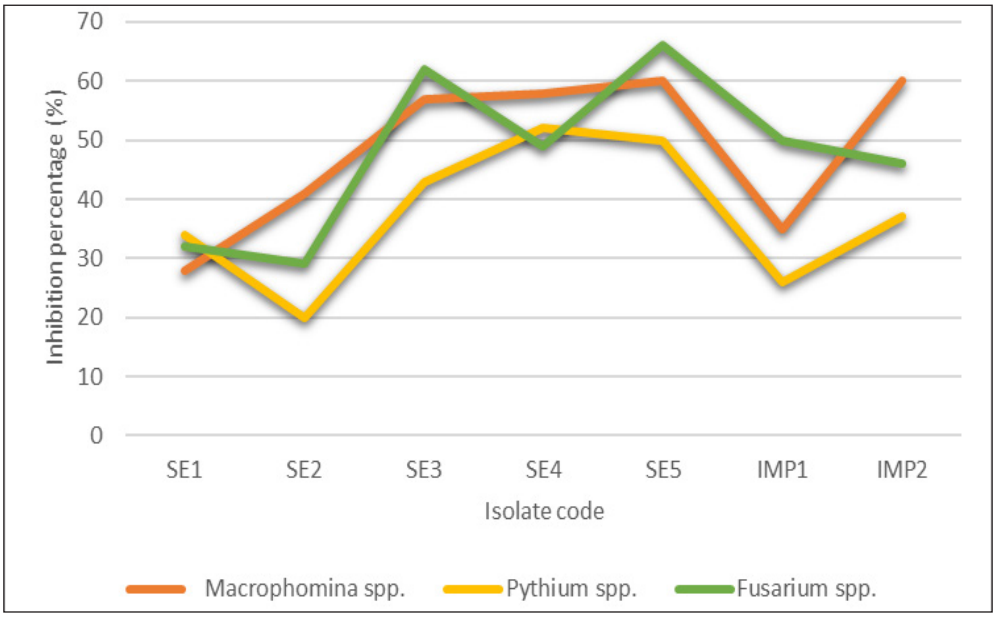

Fig. 5: Antagonistic analysis of endophytic bacterial isolates activity against several phytopathogens

P. fluroscens and *P. putida* were shown to have an effective antimicrobial activation against numerous phytopathogens, as reported by Sucher and Baker, 1982. Corresponding to research by Saritha *et al.* (2015), *P. putida* has a potent antagonistic effect against fungi *F.oxysporum*, *C. fimbriata* and *S. rolfsii*.

Chryseobacterium AD48 was believed as an anti-bacterial agent, and its vital role of antagonism against *Fusarium oxysporum*, *Fusarium culmorum*, *Fusarium solani*, *Pythium* spp. and *Rhizoctonia* spp. (Tyc *et al.* 2015). *C. indologenss* has shown a significant amount of antagonism against the bacterial phytopathogens was isolated by Hong *et al.* (2018) from root tissues of *Vigna radiata*. The endophytic bacterium encloses the bacteriocin gene clusters, which have the potential to promote plant growth.

According to Kim *et al*. (2011), *C. wanjuense* synthesized proteinase, HCN, also engaged in root colonization, swarming activity, and various similar antagonistic processes. *Chryseobacterium gleum* has been identified as a potential biocontrol agent for inhibiting *Rhizoctonia* damping off on various plants, including pepper roots (*Krause et al.* 2001).

CONCLUSION

Plant potential to promote endophytic bacterial isolates' growth was scrutinized from Coffee leaves by Leaf extraction and Imprintation method. The following isolates were tentatively identified by morphological and biochemical characterization. Among all the following PGP endophytic bacterial isolates, SE4 (*Chryseobacterium indologenss*) and IMP2 (*Pseudomonas putida*) have the maximal antagonistic effect against different pathogenic fungal species. It could be possible to have a wider spectrum of activity, enhanced level, and consistency in disease management against multiple pathogens/races of soil and airborne nature. In addition to disease control, better uptake of nutrients, improving plant growth, and enhanced crop yield can also be achieved.

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