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RESEARCH ARTICLE

Screening and Characterization of Plant Growth Promoting Endophytic Microbes of Tomato (*Solanum lycopersicum* L.) combats *Pythium aphanidermatum*

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ABSTRACT

About 45 bacteria, 9 fungi, 3 yeast, and 1 actinobacterium were isolated from plant tissues of tomato and screened for their plant growth-promoting traits and antagonistic activity against damping-off fungus *Pythium aphanidermatum*. The bacterial isolate, *Bacillus pumilus* TEB10 exhibited pathogen inhibition of about 55 and 79%, while yeast isolate *Candida tropicalis* TEY1 showed 30 and 68% in the dual plate and inverted plate assay, respectively. In addition, the *Bacillus pumilus* TB10 showed siderophore, HCN, cellulase, and phosphate solubilization activities. Similarly, yeast isolate *Candida tropicalis* TEY1 exhibited catalase, phosphate, and zinc solubilization activities. The sterol biosynthesis pathway end product ergosterol was profiled in secondary metabolites of *P. aphanidermatum*, suggesting the potentiality of this pathogenesis.

Keywords: *Tomato; Endophytes; Antagonistic activity; PGP traits*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the important vegetable crops cultivated worldwide. Pathogens infect the tomato plant at different stages and caused yield loss and sometimes plant death. Among them, one of the important nursery pathogens is *Pythium* spp., an oomycete that causes damping-off disease. The two types of damping-off, such as preemergence and postemergence, make a loss of seed germination by killing the seed before emergence, and after emergence it kills the seedling by the formation of a water-soaked lesion in the collar region. It is more widespread in the case of moist environments with high relative humidity. A tender stem with cotyledonary leaves is more susceptible to the pathogen. Reducing the inoculum in the seeding medium may minimize the disease infection. Several researches comprise of understanding different mechanisms involved in reducing the disease inoculum are the utilization of chemicals and microorganisms. These microorganisms act as biocontrol agents isolated from the phyllosphere, rhizosphere, and endophytes (Strobel, 2006). The endophytes are found to be second genome of plants and, as a whole are called as phytobiome (Lopez *et al*., 2018). Endophytes play a significant role in alleviating the biotic stress caused by pathogens.

Endophytic microbes live entire or part of their life cycle inside the tissues of living plants and cause unapparent and asymptomatic infections in plant tissues, but cause no symptoms of disease (Wilson, 1995). Similarly, endophytes are isolated from inner plant parts or from surface-disinfected tissues, which do not visibly harm the plant (Hallmann *et al*., 1997). Endophytes enter plants from different entry points and live inside without harming their host (Malfanova *et al*., 2011). They may originate from seeds and vegetative material and enter the phyllosphere and rhizosphere. They are transmitted vertically between generations by seed and vegetative material. In seeds, they can persist for years and arise during favorable conditions (Rudgers *et al*., 2009). The microbiome richness outside the host alters the endophyte population within the host. The endophytic microbiome changes even within same plant

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species with different varieties. The rich nutrients inside the plant facilitate favorable environment for endophyte's survival.

The endophytic microorganisms comprise bacteria, actinobacteria, filamentous fungi, and yeast, forming the microbiome. Cultural and molecular methods are employed for the detection, differentiation and identification of endophytes inside the host (Rosenblueth and Martinez-Romero, 2006). Among the endophytes, the population of bacteria tends to present higher specifically in root than in the shoot region (Soad A. Algam, 2005). Several endophytes are involved in plant growth promotion activities and also suppress the disease-causing pathogens associated with the host plant (Turner *et al*., 2013). The plant growth-promoting attributes include fixing atmospheric nitrogen, production of enzymes to solubilize the available nutrients, synthesis of plant hormones, production of siderophore, lytic enzymes, and secondary metabolites to inhibit the plant pathogen and to induce systemic resistance (Malusa *et al*., 2016).

Endophytes as biocontrol agents act against plant pathogenic fungi such as *R. solani*, *Pythium* sp., *Alternaria alternata*, *Fusarium* sp., *Botrytis cinerea*, *Verticillium dahlia*, *Penicillium digitatum*, *Sclerotinia sclerotiorum*, *B. fabae* and *Colletotrichum gloeosporioides* (Cao *et al*., 2005). The present study was aimed to isolate different antagonistic endophytic microorganisms against *Pythium aphanidermatum* from tomato with PGP activity.

MATERIAL AND METHODS

Surface sterilization of plant samples

Shoot and root samples of tomato (var. PKM 1) were collected from TNAU orchard and immediately used for isolation of endophytic microbes. The samples were washed in tap water to remove the adhering soil and debris and allowed to dry in shade. The plant parts were separated as shoot and root and its weight was measured. The samples were placed at laminar air flow chamber and cut into 2 cm pieces. It was first soaked in 1 % Sodium hypochlorite (NaOCl) for 1 min for external sterilization and then rinsed in sterile distilled water, followed by soaking at 70 % ethanol for 30 sec and thrice rinsed in sterile distilled water. It was allowed to dry on sterile filter paper. About 100 µL last wash water was plated in the medium for confirmation of sterility and to make sure that no epiphytic microbes associated with the sample (Cao *et al*., 2004).

Isolation of endophytes from tomato

The samples were placed in separate mortars containing 5 mL of sterile distilled water, crushed finely, and diluted serially to 10^{-3} and 10^{-4}. About 100 µL of each diluent was poured into Petri plate containing respective media for fungi (Potato dextrose agar, Rose Bengal agar), bacteria (Nutrient agar, Tryptic soy agar), yeast (Yeast extract and malt extract agar) and actinobacteria (Kenknight agar, actinomycetes agar). The plates were replicated thrice and incubated at 28±2 °C for 15 days. The colony was observed periodically, and CFU was calculated. The purified colonies were stored in glycerol stock at -80 °C.

Plant pathogen

The damping-off pathogen, *Pythium aphanidermatum* was obtained from the Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore.

Dual plate assay

The dual plate assay was conducted according to the method of Rabindran and Vidyasekaran, (1996). Mycelial plugs of 8 mm diameter of the pathogen were placed near the periphery of PDA plates, and antagonists such as bacteria, yeast, and actinobacterium were streaked respectively on the opposite end. In case of antagonistic fungi, an 8 mm mycelial plug was placed. A plate with pathogen alone served as control. These plates were inverted and maintained at 28 ± 2 °C, for five days. Percent inhibition was calculated using the formula,

$$PI = \frac{C - T}{C} \times 100$$

where PI = per cent inhibition; C = radial growth of the pathogen in the control plate; T = radial growth of the pathogen in treatment

Inverted plate assay

An 8 mm mycelial plug of the endophytic fungi was placed at the center of a 90 mm bottom Petri plate containing PDA medium. The inoculum was spread in the bottom Petri plate for endophytic bacteria and yeast isolates. The mycelial disc of the pathogen was placed on another bottom plate containing PDA. The pathogen plate was upturned and positioned over the antagonist plate. These plates were sealed with parafilm to prevent the volatile compounds from escaping. The control consisted of a pathogen plate upturned and positioned over an uninoculated PDA plate (without antagonist). These plates were incubated

at 28±2 ºC till the pathogen growth cover the entire plate. Per cent inhibition was calculated as in dual plate assay (Trivedi *et al*.,2006).

HCN production

The effective isolates of bacteria (TB10) and yeast (TEY1) were screened for the production of hydrogen cyanide. The nutrient agar media was amended with 4.4 g glycine^{-1} was inoculated with effective isolate. A Whatman no.1 filter paper soaked in 2 % sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 28±2 °C for 4 days. The development of yellow to reddish brown color indicated HCN production (Tsegaye, 2019).

Siderophore assay

Chrome Azurol Sulphonate (CAS) assay was used for the assessment of siderophore production (Schwyn and Neilands, 1987). The effective isolates of bacteria (TB10) and yeast (TEY1) were streaked in a single line on the center of the plate containing CAS medium. The un-inoculated plate act as control. These plates were incubated at 28 °C for 5–7 days for the formation of an orange zone around the bacterial colonies.

Hydrolytic enzymes activity

Pectinase Assays

Bacteria (TB10) and yeast (TEY1) were separately streaked on the Petri plate containing one fourth of nutrient agar medium amended with 0.5% pectin and incubated at 28± 2 °C. The appearance of the halo region after 2-4 days indicate pectinase positive (Tsegaye, 2019).

Cellulase assay

Bacteria (TB10) and yeast (TEY1) were streaked on the sodium carboxymethyl cellulose (CMC) amended medium and incubated for 2–3 days at 28 ± 2 °C. After incubation period, the plates were then flooded with Gram's iodine to visualize the halo zone around the colony indicates positive result (Tsegaye, 2019).

Protease assay

Proteolytic activity was observed in the medium containing skim milk. The overnight grown culture was streaked onto the medium and incubated at 28 ± 2 °C for 2-3 days. The formation of clear halo zone around the colony indicates positive for protease activity (Tsegaye, 2019).

Amylase assay

The effective isolate of bacteria (TB10) and yeast (TEY1) were streaked on soluble starch agar medium and incubated at 28 ± 2 °C for 2-3 days. The plates were then flooded with iodine solution for a minute and then poured off. Iodine reacts with starch to form a blue compound, and later it became colorless around the colony, indicating the production of amylase (Tsegaye, 2019).

Catalase assay

Petri dish containing overnight grown bacteria (TB10) and yeast (TEY1) were poured with hydrogen peroxide. The formation of oxygen molecules indicates positive reaction (Amaresan, 2012).

Zinc and Phosphate solubilization

The overnight grown culture was spot inoculated on the zinc and phosphate solubilization medium and incubated at 28 ºC. The appearance of halo region shows a positive effect for solubilization.

Collection and extraction of non- volatile soluble metabolites

Pythium aphanidermatum was grown in PD broth in three replicates and incubated at 28 ± 2 ºC for 7 days. An uninoculated PD broth served as control. After passing through muslin cloth, the culture filtrate was centrifuged at 6000 rpm for 15 min, mixed with equal quantity of ethyl acetate, and incubated overnight under shaking conditions. The solvent phase was separated and concentrated in a vacuum flash evaporator. After sufficient drying, the crude metabolites were extracted using 1 mL methanol and filtered through a 0.2 µm bacteriological membrane filter for GC-MS analysis (Meena, 2017).

Non- volatile soluble metabolites analysis by GC-MS

The purified crude methanolic extract was subjected to GC-MS analysis in a Perkin Elmer GC-MS Clarus® SQ 8 equipped with DB-5MS (Agilent, USA) capillary standard non-polar column with dimensions 0.25 mm OD x 0.25 µm ID x 30 m length. The instrument was set to an initial temperature of 40˚C, and the injection port temperature was ensured at 220 ˚C, interface temperature set 250 °C, source kept at 220 °C, oven temperature-programmed as 75 °C for 2 min, 150 °C @ 10 °C/min, up to 250 °C at 10 °C per min. The GC conditions were: 1:12 split, helium carrier at 20 psi. The MS conditions were: positive ion mode, electron impact spectra at 70 eV.

The mass spectral scan range was set at 50 to 600 Da. The MS peaks were determined by their scatter pattern. The linear regression coefficient was used to calculate the concentrations in the samples from peak areas obtained in the chromatographs. The bioactive molecules were identified by comparison of mass spectra with NIST 08 Mass Spectra Library (National Institute of Standards and Technology). The name, molecular weight, and structure were ascertained from NIST, PubChem, and HMDB databases (Leylaie and Zafari, 2018).

Statistics analysis

All the experiments were analyzed independently. The treatment means were compared by Duncan's multiple range test (DMRT) Gomez *et al.* (1984). All the data were analyzed statistically by AGRESS and interpreted.

RESULTS AND DISCUSSION

Morphology of P. aphanidermatum

The microscopic observation of *Pythium aphanidermatum* revealed aseptate and colorless hyphae with the presence of antheridia and oogonia. This sexual reproductive structure observed was similar to the *P. aphanidermatum* causing damping-off in Cucumber Priyanka *et al*. (2019) (Figure 1a, b). The pathogenicity test on tomato showed the symptoms of shrinking of the stem near the collar region and toppling down of seedling due to rotting. (Figure 1c).

Isolation of endophytic microorganisms

Endophytic microorganisms were isolated from the root and shoot region of the tomato to a total of 45 bacteria, 9 fungi, 3 yeast, and 1 actinobacterium registering maximum endophytes from the root region (Table 1). All the isolated endophytes were differentiated based on morphology (Table 2). Bacteria were considered as the major group of endophytes in the tomato plants, with five major phyla considering Proteobacteria was the most represented (90 %), followed by Actinobacteria (1.5 %), Planctomycetes (1.4 %), Verrucomicrobia (1.1 %), and Acidobacteria (0.5 %) (Romero *et al*., 2014). Sen *et al*, (2019) isolated yeast endophyte *Rhodotorula mucilaginosa* JGTA-S1 from *Typha angustifolia* root. El-Tarabily *et al*. (2008) isolated 4.56 $\log_{10}$ CFU g^{-1} of actinobacteria from the fresh root of Cucumber and observed that the colonization was more in the root than in the shoot region.

Screening of endophytes against damping-off pathogen

The isolated endophytes were screened for their antagonistic property against *P. aphanidermatum* by dual plate and inverted plate assay. Among the 45 bacteria, nearly 17 were effective against the damping-off pathogen with 50 to 70 % inhibition. The bacterial isolate TEB11 exhibited a maximum inhibition of 61.66 % in the dual plate assay, and the isolate TEB10 inhibited the pathogen by 79.28 % in the inverted plate assay. The dual plate assay is the direct interaction of both organisms, whereas inverted plate assay is the indirect effect of volatile mediated inhibition. The inverted plate assay registered a maximum efficiency for inhibiting pathogen growth (Table 3). Priyanka (2019) recorded an inhibition of *P. aphanidermatum* at about 40 %, and 36 % by endophytic bacteria such as *Pseudomonas aeurginosa* (MPP EB 4 and MPP EB 3) and *Achromobacter denitrificans* (MPP EB 8), respectively. Similarly, Lopez *et al*. (2018) observed that among 11 seed endophytic bacteria, only six (4 *Bacillus* sp., *Micrococcus* sp., *Paenibacillus polymyxa*) bacteria efficiently inhibited the growth of pathogens *viz*., *Alternaria* sp., *Stemphylium* sp. and *Corynespora* sp. Among the 6 species, only three strains of *Bacillus* sp. inhibited the mycelial growth by both direct and volatile mediated interaction. Muthukumar *et al*. (2010) observed that the endophyte of chilli, *Pseudomonas fluorescens* EBS 20 inhibited the damping-off pathogen by 76 % with wide inhibition zone in a dual plate assay.

Among the 9 endophytic fungi screened for its efficiency, it was observed that the growth of the pathogen *P. aphanidermatum* was faster than that of the endophytes. Hence, the inhibition was between 10 to 25 % in the dual plate assay and less than 15 % in the inverted assay. The endophytic fungi, *Aspergillus terreus* inhibited the *P. aphanidermatum* with an inhibition zone of 8.7mm and reduced the spore production (Halo *et al*., 2018). Vinayarani and Prakash, (2018) found that endophytic *T. harzianum* TharDOB-31 inhibited the damping-off fungi, *P. aphanidermatum* registering 70 % inhibition.

In dual plate assay, the endophytic yeast inhibited the pathogen growth of up to 30 % inhibition. Whereas, the inverted plate assay demonstrated up to 68% inhibition exhibiting changes in the morphology of the mycelia. While other yeast isolates such as TEY2 and TEY3 didn't inhibit pathogen growth effectively. In inverted plate assay, when the exposure of volatile was removed,

the pathogen gets back to its normal growth, suggesting that the volatile act as a fungistatic rather than fungicidal. A similar result was obtained for the rhizospheric yeast *Torulaspora globosa* DMKU-RP31 which produce significant volatile compounds that inhibited the plant pathogens *Fusarium moniliforme*, *Helminthosporium oryzae* and *Rhizoctonia solani* (Nutaratat *et al*., 2014). The endophytic yeasts such as *Saccharomyces, Torulaspora*, and *Debaryomyces* isolated from *Tagetes erecta*, *Azadirachta indica* and *Carica papaya* inhibited *Fusarium solani*, *F. oxysporum* and *M. phaseolina* (Fareed *et al*., 2019).

The effective isolate among the bacteria against *P. aphanidermatum* was *Bacillus pumilus* TEB10, which showed maximum inhibition in volatile mediated interaction of about 79 % and also recorded significant inhibition in a dual plate assay. Among the yeast, the isolate *Candida tropicalis* TEY1 was effective in inhibiting the pathogen growth in volatile-based interaction than in dual plate (Figure 2). Since indirect interaction is marked effective than direct interaction, the volatile based biocontrol mechanisms may attribute efficient management strategy. Amaresan *et al*. (2012) observed that, among the 82 endophytic bacteria, the isolates BECS3, BECS6, and BECS7 showed multiple attributes and demonstrated plant growth promotion properties through a tomato and chili-based bioassay under greenhouse conditions.

Identification of effective isolate

The PCR analysis of endophytic yeast TEY1 resulted the amplicon size of around 600bp and showed 99.8 % similarity with *Candida tropicalis* whereas the effective bacterial isolate TEB10 resulted the amplicon size of 1500bp and showed 99.8 % similarity with *Bacillus pumilus* when compared with isolates deposited in NCBI database.

Plant growth-promoting traits

The effective bacterial isolate *B. pumilus* TEB10, showed positive for siderophore activity by changing the blue background to an orange halo around the bacterial colony whereas *C. tropicalis* TEY1 did not show siderophore activity. Lopez *et al*. (2018) observed that seed endophytic bacteria *Bacillus* sp. and *Acenitobacter* sp. produce siderophore. Protease activity was observed for *B. pumilus* TEB10 by the formation of the halo zone around the colony but not in *C. tropicalis* TEY1.

Catalase activity was detected for the yeast *C. tropicalis* TEY1 by the formation of oxygen when H_2O_2 was added to the culture. The bacterium (*B. pumilus* TEB10) showed positive for HCN and cellulase activity whereas the yeast *C. tropicalis* TEY1 was devoid of HCN and cellulase. *B. pumilus* TEB10 showed positive for phosphate solubilization, while *C. tropicalis* TEY1 did not solubilize phosphate (Table 4 and Figure 3). Amaresan *et al*. (2012) isolated 82 bacterial endophytes in which many produced siderophore, solubilized phosphate, and not all isolate exhibited amylase, protease, cellulase, and lipase activities. Nutaratat *et al*. (2014), observed that the endophytic yeast from rice and sugarcane solubilize phosphate, zinc, and showed catalase positive. *Rodotorula mucilaginosa* isolated from *T. angustifolia* solubilize calcium phosphate and improved rice growth when inoculated in low phosphate media (Sen *et al*., 2019). Whereas in the case of zinc solubilization, the bacteria reacted negative, and yeast reacted positive. Abdallah (2018) reported that the isolated endophytic bacteria solubilize phosphate, while some strains, such as *Pseudomonas geniculata* CT19 (KR818061) and *Bacillus subtilis* SV5 (KR818065) did not solubilize phosphate.

Profiling secondary metabolites produced by pathogen

The compounds present in the ethyl acetate fraction of *P. aphanidermatum* was Thieno[2,3-d]pyrimidine-6-carboxylic acid, 5-; methyl-4-oxo-3-[2-(pyrrolidin-1-yl)ethyl]-2-thioxo-1; L-Pipecolic acid; furan-3-one; [(2-propenyloxy)methyl]- Oxirane; Thymine; 4H-Pyran-4-one; Dodecanoic acid; Methyl myristoleate; Methyl tetradecanoate; 12-Methyl-oxa-cyclododecan-2-one; Linolenic acid; Methyl stearate; 9,12-Octadecadienoyl chloride; Eicosatrienoic acid; Ergosterol; Docosanoic acid (Table 5 and Figure 4). These metabolite compounds might be responsible for the induction of disease in plants and for the growth and survival of the pathogen. The compound ergosterol is absent in the oomycetes, which is the target site for many fungicides. The lack of complete set of genes in sterol biosynthetic pathway made the pathogen resistant to fungicides. But in this study the GC-MS analysis showed the presence of sterol compound. Lerksuthirat *et al*. (2016) showed that ergosterol, the primary constituent in cell membrane, was present only in true fungi such as *Candida albicans* and *Aspergillus fumigatus.* It was absent in Pythiales (*Pythium insidiosum*) and Peronosporales (*Phytophthora* sp.). This absence of sterol act as chemotaxonomic marker for the oomycetes phylogenetically.

In this study, ergosterol was profiled in the metabolites of *P. aphanidermatum* at same retention time and mass value compared to true fungi. Trigos *et al.* (2005) reported for the first time that the Peronosporales genus *Phytophthora drechsleri* produced ergosterol.

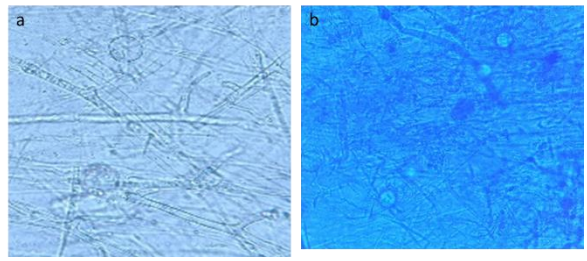

Figure 1. Morphological and pathogenicity features of *P. aphanidermatum*

a. Presence of aseptate mycelia with antheridium and oogonium b. Presence of oogonium (Lactophenol mount) c. Pathogenicity in tomato – shrinking

Table 1. Endophytes present in the tomato shoot and root region

Endophytes	Shoot	Root
	cfu/gram sample	
Bacteria	Numerous	Numerous
Fungi	6	3
Yeast	1	2
Actinobacteria	0	1

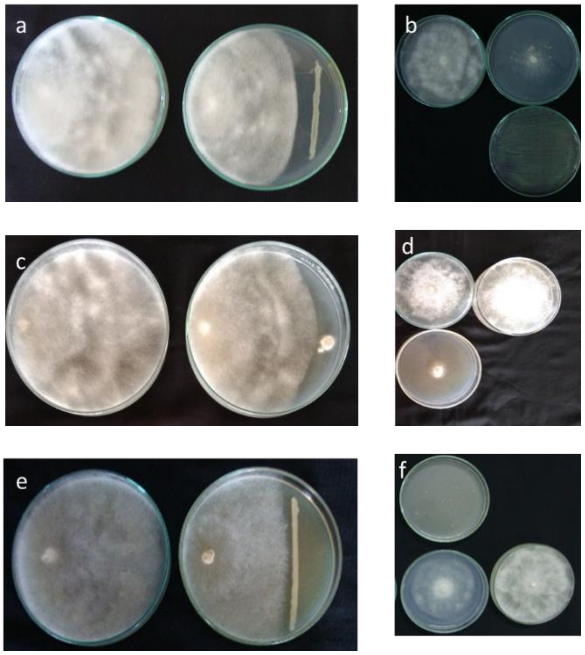

Figure 2. Antagonistic effect of endophytes in dual and inverted plate assay Dual plate and inverted plate assay. a, b – Bacteria TEB10 with *P. aphanidermatum*. c, d – Fungi with *P. aphanidermatum*. e, f - Yeast TEY1 with *P. aphanidermatum*

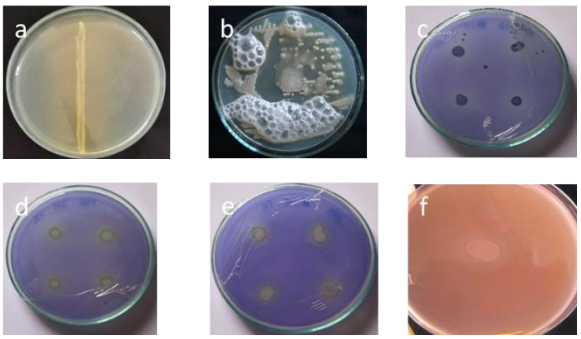

Figure 3. Plant growth promotion traits present in endophytes

a. Siderophore activity - change in color from blue to orange by bacterium TEB10, b. Catalase activity – evolution of oxygen by adding H_2O_2 by yeast TEY1, c, d. Phosphate solubilization – formation of halo zone around the colony by bacterium TEB10 and yeast TEY1, e. Zinc solubilization – formation of halo zone by yeast TEY1, f. Cellulase activity – formation of halo zone by bacterium TEB10

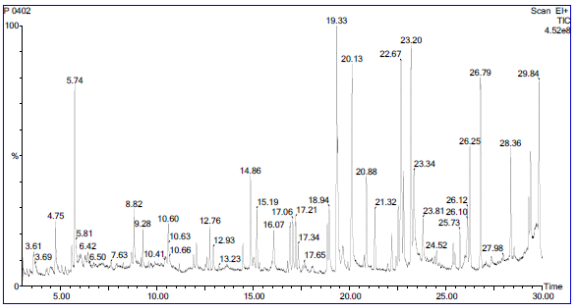

Figure 4. Chromatogram of *P. aphanidermatum*

Table 2. Morphology of isolated endophytes

Name	Morphology
Fungi	
TEF1	Mycelia is cream with black spots in ventral side and hypha septate.
TEF2	Mycelia is light brown with green spores, pink pigmentation in ventral side and septate hypha
TEF3	Mycelia is grey, aseptate hypha, fluffy and presence of black spots in ventral side
TEF4	Mycelia is white and fluffy with pink rings in ventral side with aseptate hypha
TEF5	Mycelia is white, fluffy growth with septate hypha
TEF6	Mycelia is white to cream color with aseptate hypha
TEF7	Mycelia is white with green spores and the hypha is septate
TEF8	Mycelia is white with small pycnidia like structures with aseptate hypha
TEF9	Mycelia is black with orange pigmentation in ventral side with aseptate hypha
Actinobacteria	
TEA1	Grey color colony with chain of spores.
Yeast	
TEY1	White to cream colony, cell is round with budding
TEY2	Orange colony, round cell
TEY3	White colony, round cell
Bacteria	
TEB10	Initially green, later become cream with striations, rod shaped cell.

Table 3. Effective bacterial endophyte against *P. aphanidermatum*

Bacterial isolate	Per cent inhibition	
	Dual plate	Inverted plate
TEB1	52.77^{fg}	50^{h}
TEB2	52.21^{f}	44.4^{i}
TEB3	59.44^{b}	60d^{e}
TEB4	57.77^{bc}	61.11^{d}
TEB5	56.1^{cde}	60.55^{d}
TEB6	50^{g}	66.5^{b}
TEB7	54.5^{de}	56.66^{f}
TEB8	49.44^{g}	67.77^{b}
TEB9	52.22^{f}	50^{h}
TEB10	55.11^{de}	79.28^{a}
TEB11	61.66^{a}	58.33^{ef}
TEB12	55.55^{de}	53.88^{g}
TEB13	55.55^{de}	58.33^{ef}
TEB14	50.55^{fg}	61.66^{d}
TEB15	56.66^{cd}	57.21^{f}
TEB16	54.44^{e}	64.44^{c}
TEB17	52.22^{f}	50^{h}
Control	0.00^{h}	0.00^{i}
Sed	0.1369	0.1329
CD (0.05)	0.2783	0.2703

Table 4. Plant growth promotion traits present in endophytes

Assay	Bacteria TEB10	Yeast TEY1
Siderophore	+	-
HCN	+	-
Catalase	-	+
Cellulase	+	-
Protease	-	-
Pectinase	-	-
Zinc solubilization	-	+
Phosphate solubilization	+	+

(+ indicates positive: - indicates negative reaction)

Table 5. Secondary metabolites present in *P. aphanidermatum*

Retention time	Compounds	Area percent
3.058	Thieno [2,3-d] pyrimidine-6-carboxylic acid	0.745
3.269	L-Pipecolic acid	0.735
3.424	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	0.703
3.614	Oxirane, [(2-propenyloxy) methyl]-	1.928
4.754	Thymine	1.145
5.744	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	3.741
14.468	Methyl myristoleate	0.62
14.863	Methyl tetradecanoate	2.047
17.059	12-Methyl-oxa-cyclododecan-2-one	1.009
17.214	Pentadecanoic acid, methyl ester	0.859
18.99	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	1.267
22.191	ç-Linolenic acid, methyl ester	0.793
23.196	Methyl stearate	4.33
23.351	9,12-Octadecadienoyl chloride, (Z,Z)	2.486
25.727	8,11,14-Eicosatrienoic acid, methyl ester	0.762
28.363	Heneicosanoic acid, methyl ester	1.838
29.684	Ergosterol	1.365

Conclusion

The present research stated that different endophytic microorganisms were residing inside the plant with plant growth-promoting and antagonistic traits. Among the isolated endophytic microorganisms, a bacterium (*B. pumilus* TEB10) and a yeast (*C. tropicalis* TEY1) exhibited effective antagonistic activity and PGP traits. These effective endophytes may be used as bioinoculant in combination with alleviating biotic stress and plant growth promotion.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

Authors should ensure that they have written and submit only entirely original works, and if they have used the work and/or words of others, that this has been appropriately cited. Plagiarism in all its forms constitutes unethical publishing behavior and is unacceptable.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail;

Author contributions

Research grant - US, Idea conceptualization - US, Experiments - APS, Guidance - US, GK, writing original draft - APS, Writing- reviewing & editing - APS, US, GK.

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