Phosphate solubilization, siderophore production and extracellular enzyme production activities of endophytic fungi isolated from tea (*Camellia sinensis*) bushes of Assam, India

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Abstract

In the present study, a total of ten endophytic fungi were isolated from Camellia sinensis root, stem and leaf samples collected from twelve different tea gardens of Assam, India and evaluated for phosphate solubilization, siderophore production and extracellular enzyme producing activities. Out of ten, nine fungi were found positive for phosphate solubilization, siderophore production and cellulase activities. The highest fungal population was recorded in the root sample ORG-B (7.5 x 10^5) followed by ORG-A (7.4 x 10^5). Phosphate solubilization index was recorded highest in Penicillium sclerotiorum (2.87±0.01) followed by Penicillium sp F 7 (2.22±0.02) after 9 days of incubation.

The highest siderophore percentage was observed in P. chrysogenum F 5 (61.09 ± 0.75%) followed by P. chrysogenum F1(56.29 ± 0.43) after 6 days of incubation. The highest cellulase (42.25±2.6 µg/ml/30 min), amylase (41.50±1.54 µg/ml/30 min) and xylanase (32.00±2.18 µg/ml/30 min) activites were recorded for P. chrysogenum F5, Aspergillus fumigates and A. niger respectively.

Keywords: *Endophytes, biofertilizer, siderophore, extracellular enzyme, phosphate solubilizer.*

Introduction

Endophytes colonize inside any part of the plant tissues without causing substantial harm to the host[6]. They may reside for a specific period or may spend their whole life inside the host plant tissues. Sometimes, some pathogenic microbes may also be present inside the plant body without showing pathogenicity. In such cases, the latent pathogen may also be considered an endophyte[10,38]. Though different microbes may colonize plant tissues, endophytes generally represent bacteria and fungi. They are ubiquitous in their presence irrespective of their place of origin and are responsible for exerting some beneficial activities to the host[14]. The role of endophytes in plants ranges from the protection of the plants against herbivores to improve in mineral absorption and stress tolerance[4]. Phosphorus (P) is one of the major essential elements required for plant growth and development. It is the essential macronutrient next to nitrogen (N) required for plant growth and development within the optimum concentration[31]. The present-day agriculture system is mostly dependent on chemical phosphate fertilizers which are costly and negatively impact soil health[22]. More than 95% of soil phosphate is found in insoluble forms like phosphate of aluminium, calcium and iron. A major part of the applied phosphate chemical fertilizers is also converted into insoluble forms depending on the soil pH.

Hence, very little amount of phosphate in soil remains available for plant uptake[42]. The use of rock phosphates instead of other chemical phosphate fertilizers is considered one of the best and most cost-effective ways. However, the solubility of rock phosphate is very limited in normal and low acidic soil conditions[15].

Presently, the best and most cost-effective way to make phosphate available to the plant is the soil inoculation of phosphate solubilizing microorganisms[33]. Phosphate solubilizing fungi in soil constitute about 0.1–0.5% of the total fungal populations where the endophytic fungal population occupies a significant part. Endophytic fungi are representative of the rhizosphere fungal population. It is now well established that the endophytic microbial population is highly dependent on the rhizosphere population and the host specificity[8].

The main mechanism of phosphate solubilization by the rhizosphere and endophytic fungi is the production of acid phosphatases and organic acids like gluconic acid[41]. The major endophytic P-solubilizing fungi belong to the genera *Penicillium*, *Aspergillus*, *Piriformospora*, *Curvularia*, *Fusarium*, *Cladosporium*[2,8].

Siderophores are some low molecular weight compounds having very high affinity to Fe^{3+} and are generally secreted by microorganisms, both aerobic and facultative anaerobic under iron stress conditions[30]. They have molecular weight less than 1000 Dalton and can easily bind to iron even when the metal is present only in trace. Production of siderophores by rhizosphere and endophytic microbes is one of the major plant growth promoting traits[16]. The first report of siderophore production by fungi was reported by Neilands[28] in 1952 in *Ustilago sphaerogena*. After that several

microbes are reported for siderophore production which competes with other microbes and chelate iron in iron starved condition making them available for plants[18].

Like the fungal communities of the rhizosphere, endophytic fungi produce extracellular enzymes like amylase, pectinases, cellulases, xylanase, lipases etc.[17,39] They secrete these enzymes to obtain their nutrition from the host as well as a resistance mechanism against pathogenic invasion.

Mohini et al[25] isolated *Cladosporium* sp., *Rhizoctonia* sp., *Aspergillus* sp., *Chaetomium* sp., *Biosporu*s sp., *Fuzarium* sp., *Curvularia* sp., *Cladosporium* sp. and *Colletotrichum* sp. with potential extracellular enzyme-producing activity from some medicinal plants.

Lumyong et al[20] isolated and characterized 13 different species of endophytic fungi from *Camellia sinensis, Cinnamomum iners, Garcinia cowa, Litsea salicifolia, Manglietia garrettii* and *Trichilla connaroides* and reported that all the isolated fungal endophytes were potent for producing cellulases, mannanases and xylanases in both their prepeproductive and reproductive stages.

Besides the enzyme-producing activity, endophytic fungi are well known for the production of antibacterial, anticancer, antioxidants, anti-diabetic and immunosuppressive compounds[32]. *Pestalotiopsis microspore* is a common rainforest endophyte and has extensive biochemical diversity and hence is a source of various bioactive compounds like ambuic acid, a potent antifungal compound that is now reported in many other endophytic fungi[19,37].

Keeping the view of importance of phosphate solubilizing and siderophore producing microbes in sustainable agriculture, this study aimed to isolate endophytic fungi with phosphate solubilization and siderophore production potential from tea, a major crop of India along with the potential to produce different extracellular enzymes.

Material and Methods

Collection of samples: Healthy and disease-free young root, stem and leaf samples were collected from mature tea (*Camellia sinensis*) shrubs from different tea gardens of Upper Assam, India. Collected samples were packed immediately in sealed polybags, kept in the ice box and carried to the laboratory for further use.

Sample preparation and isolation of endophytic fungi: Young root, stem and leaf samples were washed separately with tap water to remove the soil adhering to the roots and the debris attached to the stem and leaves followed by washing with distilled water five times. After cleaning, five grams of root sample were dipped in 70% ethanol for five minutes, washed with sterilized distilled water and again dipped in 0.1% $HgCl_2$ for one minute. However, an equal amount of stem and leaf samples were treated with 70% ethanol and 0.05% $HgCl_2$ for one minute. The effect of the surface sterilizing agents was removed by repeated washing of the samples with sterilized distilled water ten times[5].

The efficacy of the surface sterilization was confirmed by rolling a piece of sample on a nutrient agar plate and incubated for 72 hours. No microbial growth confirms successful surface sterilization. After proper surface sterilization and removal of surface sterilizing agents, root, stem and leaf samples were crushed separately to prepare a tissue homogenate which was further suspended in 45 ml of sterilized distilled water and then diluted serially following the standard procedure of serial dilution. 0.1 ml of serially diluted tissue homogenate was spread in Czapeck dox agar (CDA) medium plates and incubated for 10 days at 25^0C. The process was carried out in front of laminar air flow to avoid contamination.

The fungal population was estimated in terms of colony forming units (CFU) after 10 days of incubation. Pure culture of the fungal isolates was done by using the single spore technique described by Choi et al[7].

Screening of phosphate solubilization: Qualitative estimation of phosphate solubilization activity of the isolates was carried out periodically in Pikovskya's medium plates. Isolates showing clear zones around the growing colonies were considered positive for P solubilization[9,27]. The solubilization index was calculated by using the standard formula:

$$\text{Phosdphate solubilization index} = \frac{\text{Diameter of the halo zone}}{\text{Colony diameter}}$$

Total diameter = (Colony diameter + halo zone)

Screening and estimation of siderophore production: Screening of siderophore production by endophytic fungal isolates was carried out using chrome azurol S (CAS) agar medium[3]. The CAS agar medium was a mixture of four different solutions that were prepared and sterilized separately before mixing. Solution I (Fe-CAS indicator solution) consisted of 10 ml of 1mM $FeCl_3.6H_2O$ (in10 mM HCl), 50 ml of aqueous solution of CAS (1.12 mg/ml) and 40 ml of aqueous solution of hexadecyl trimethyl ammonium bromide (1.82 mg/ml). Solution II (buffer solution) was prepared by dissolving 30.24 g of PIPES (peperazine-N-N-bis-2-ehansulphonic acid) in 750 ml of distilled water in 0.8% salt solution to make the volume 800 ml., adjust the pH at 6.8 with 50% KOH. Solution II was autoclaved after adding 15 grams of agar. Solution III contained 2 g glucose, 2 g mannitol and trace elements in 70 ml distilled water. Solution IV was 30 ml of filtered and sterilized 10% casamino acid.

After autoclaving, solution III and IV were added to the buffer solution (solution II) and at last solution I (indicator) was added with sufficient stirring to mix the indicator well

and poured into Petri dishes in front of the laminar air hood. Actively growing fungal cultures were inoculated on CAS agar medium plates and incubated for one week at 25ºC. Orange haloes around colonies were considered for siderophore production.

Quantitative estimation of siderophores production was determined by using CAS- shuttle assay[29]. In this method, 0.5 ml of iron starved culture supernatant was mixed with 0.5 ml of CAS reagent (Solution I) and absorbance was measured at 630 nm against a reference containing 0.5 ml of uninoculated broth and 0.5 ml of CAS reagent. Siderophore content in the aliquot was determined by using the following formula:

$$\% \text{ of siderophore units} = \frac{Ar - As}{Ar} \text{ X } 100$$

where Ar = absorbance of reference at 630 nm and As = absorbance of samples at 630 nm.

Screening of isolates for amylase production: To screen out the amylase-producing endophytes, isolates were grown on starch agar plates (2% soluble starch) and incubated for 72 hrs at 28ºC for their proper growth. After incubation, plates were flooded with Gram's iodine solution. Iodine reacts with starch to produce a blue-colored complex. The clear zone around the colony indicated amylase production and starch hydrolysis[36]. The diameter of the clear zone and the colony were noted down.

Screening of isolates for Pectinase production: For screening of fungal pectinase activity, Czapekdox agar with 1% pure pectin was used as the sole source of carbon. Isolates were inoculated and incubated for 72 hours at 28ºC. After incubation, plates were flooded with potassium iodide solution and observed the formation of clear zones around the colonies. The diameters of the clear zone and the colony were noted down.

Screening of isolates for xylanase production: Xylanase production of endophytic microbial isolates was screened by modifying the method of Soares et al[34] as mentioned for pectinase production. Here, 1% xylan from blisswood was used in place of pectin as the sole source of carbon. Isolates were inoculated and incubated for 72 hours at 30ºC for their proper growth. After incubation, plates were flooded with potassium iodide solution and observed the formation of clear zones around the colonies. Minimal salt agar plates amended with 0.1% carboxymethyl cellulose (CMC) were inoculated with freshly growing fungal cultures. The plates were incubated at 28ºC for 72 hours. Following incubation, plates were flooded with Gram's iodine solution and a clear zone was observed around. The zone diameter was measured[13].

Estimation of extracellular enzyme activity: Quantitative estimation of extracellular enzyme producing activity of the fungal isolates was carried out by using the simple DNS (dinitosalicylic acid)-spectophotometic method[24].

Molecular approaches for fungal identification

Extraction of Fungal genomic DNA: Isolation of fungal genomic DNA was done from actively growing fungal colonies. Collected fungal hyphae were ground with sterilized sand and 400 µl of fungal lysis buffer (tris HCl-50 mM; EDTA-50 mM; SDS- 3%; ß- mercaptoethanol 1% just before use) in 2 ml Eppendorf tubes. Centrifuged the mixture at 4ºC in a cooling centrifuge in 12,000 rpm for 10 minutes. Mixed the supernatant with 300 µl of phenol and 300 µl of chloroform: isoamyl alcohol (24:1). Mixed well; centrifuged at 4ºC at 12000 rpm for 10 minutes.

Collected the supernatant and suspended in 500 µl of 99.9% (v/v) chilled isopropanol. Mixed well; stored at minus 20ºC for overnight. Centrifuged and discarded the supernatant, washed the pallet with 70% ethanol, air dry in front of laminar airflow and dissolved the pallet in 30 µl tris- EDTA (1 mM tris HCl and 1 mM EDTA with pH 8.0). Added 2 µl of RNAse, mixed well and loaded in 0.8% agarose gel electrophoresis to confirm the presence of DNA. DNA concentration in the solution was determined with the help of nano-drop DNA quantifier[26].

Amplification of fungal ITS region: PCR amplification of fungal ITS (internal transcribed spacer) was carried out using universal ITS 1 (5´ CCG AAT TCG TCG ACA ACC TGG TTG ATC CTG CCA GT 3´) and ITS 4 (5´ CCC GGG ATC CAA GCT TGA TCC TTC TGC AGG TTC ACC T 3´) primer sequences as forward and reverse primer respectively. Amplification was carried out for 35 cycles of PCR profile with 94ºC for 3 min, 94ºC for 1 min, 50ºC for 30 s, 72ºC for 1 min and 72ºC for 7 min 4ºC for infinity. Amplification of ITS was confirmed by 1.2% agarose gel electrophoresis and the concentration of DNA was determined by using a nano-drop DNA quantifier[26]. After sequencing, the isolates were identified and the sequences were submitted to Genbank, NCBI and obtained the accession numbers.

Results

Population of endophytic fungi: The fungal population of root, stem and leaf samples was assessed in terms of colony forming units (CFU). It was observed that the root samples harbour higher population of fungi in comparison to stem and leaf. The fungal population ranged between 3.4 x 10^4 (DOR) and 7.5 x 10^5 (ORG-B) among the root samples; 2.3 x 10^2 (DOR) and 2.8 x10^3 (ORG-A) among stem samples; and 1.2 x 10^2 (DOR) and 2.5 x10^3 (ORG-B) among the leaf samples. Among the root samples, the highest CFU was observed in the sample ORG-B (7.5 x 10^5) followed by ORG-A (7.4 x 10^5). The fungal population in stem samples was found to be highest in ORG-A (2.8 x10^3) followed by ORG-B (2.6 x 10^3). However, among the leaf samples, the highest fungal population was observed in ORG-B (2.5 x10^3) followed by ORG-A (2.3 x 10^3; table 1). A total of 10 different endophytic fungal isolates were isolated from tea bushes based on their morphology, out of which 9 were tested positive for phosphate solubilization and were subjected to further analysis.

Phosphate solubilization: Periodic estimation of phosphate solubilizing activity of the isolates was carried out by calculating the solubilization index in Pikovskaya's agar plates. All the 9 fungal isolates exhibited remarkable phosphate solubilizing activity which ranged between 1.07±0.01 and 1.80±0.02 after 3 days; 1.21±0.01 and 2.66±0.06 after 6 days; 1.4±0.04 and 2.22±0.02 after 9 days and between 1.31±0.01 and 2.63±0.04 after 12 days of incubation. The highest phosphate solubilizing activity was observed in *P. sclerotiorum* (2.87±0.01) followed by *Penicillium sp* F 7 (2.22±0.02), *A. niger* (2.06±0.01) and *P. chrysogenum* F7 (1.98±0.05 *5*) after 9 days of incubation. Decrease in phosphate solubilization index was observed beyond 9 days of incubation, (table. 2, fig. 1; fig. 3A).

Siderophore production: Both qualitative and quantitative estimation of siderophore producing activity of endophytic fungal isolates of tea bush were carried out by using CAS assay method. The diameter of the orange halo zones in CAS agar culture of the isolates ranged between 6.10 ± 2.07 mm and 12.78 ± 2.18 mm after 6 days of incubation. In the qualitative estimation, the highest siderophore activity was recorded for *P. chrysogenum* F5 (12.78 ± 2.18 mm) followed by *P. chrysogenum* F1 (12.33 ± 2.08 mm), *Penicillium sp* F 7 (9.11 ± 1.08) and *P. crustosum* (9.06 ± 1.92) after 6 days of incubation.

Amount of siderophores released to the iron starved culture supernatant was carried out by CAS shuttle assay. The siderophore percentage in the culture supernatant ranged between 32.31 ± 0.7% (*A. niger*) and 61.09 ± 0.75% (*P. chrysogenum F 5*) after 6 days of incubation. The highest siderophore percentage was observed in *P. chrysogenum F 5* (61.09 ± 0.75%) followed by *P. chrysogenum* F1(56.29 ± 0.43), *Penicillium sp* F 7 (49.48 ± 2.38), *P. crustosum* (47.27 ± 0.35) and *P. sclerotiorum* (42.28 ± 1.60) after 6 days of incubation (table 3, fig. 2; fig. 3 B and C).

Table 1
Fungal population in tea root, stem and leaf samples.

Sample	Fungal CFU		
	Root	**Stem**	**Leaf**
AAU	6.6×10^4	2.6×10^2	1.5×10^2
ENG	4.2×10^4	2.7×10^2	1.5×10^2
CTE	3.6×10^4	2.4×10^2	1.4×10^2
BNCA	6.5×10^4	2.6×10^2	1.5×10^2
JTE	6.2×10^4	2.4×10^2	1.6×10^2
BTE	6.0×10^4	2.5×10^2	1.3×10^2
PTE	5.0×10^4	2.4×10^2	1.5×10^2
KTG	5.2×10^4	2.6×10^2	1.6×10^2
DOR	3.4×10^4	2.3×10^2	1.2×10^2
ORG-A	**7.4×10^5**	**2.8×10^3**	**2.3×10^3**
ORG-B	**7.5×10^5**	**2.6×10^3**	**2.5×10^3**
HTE	7.2×10^4	2.8×10^2	2.0×10^2

Table 2
Periodic Phosphate solubilization activity of endophytic fungi.

Fungal endophytes	Phosphate solubilization index			
	DAY 3	**DAY 6**	**DAY 9**	**DAY 12**
Penicillium chrysogenum F1	1.34±0.05	1.56±0.02	1.74±0.03	1.61±0.01
Aspergillus fumigatus	1.27±0.03	1.46±0.01	1.62±0.01	1.51±0.01
Penicillium crustosum	1.25±0.16	1.49±0.005	1.54±0.01	1.34±0.02
Aspergillus niger	1.64±0.09	1.97±0.01	2.06±0.01	1.87±0.01
Penicillium chrysogenum F5	1.30±0.11	1.71±0.01	1.98±0.05	1.62±0.01
Penicillium sp F7	1.67±0.04	2.04±0.02	2.22±0.02	1.93±0.02
Cladosporium gossypicola	1.07±0.01	1.21±0.01	1.4±0.04	1.31±0.01
Penicillium sclerotiorum	1.80±0.02	2.66±0.06	2.87±0.01	2.63±0.04
Fusarium sp.	1.44±0.02	1.68±0.02	1.79±0.01	1.65±0.01

Values are mean ± SD of five replicas

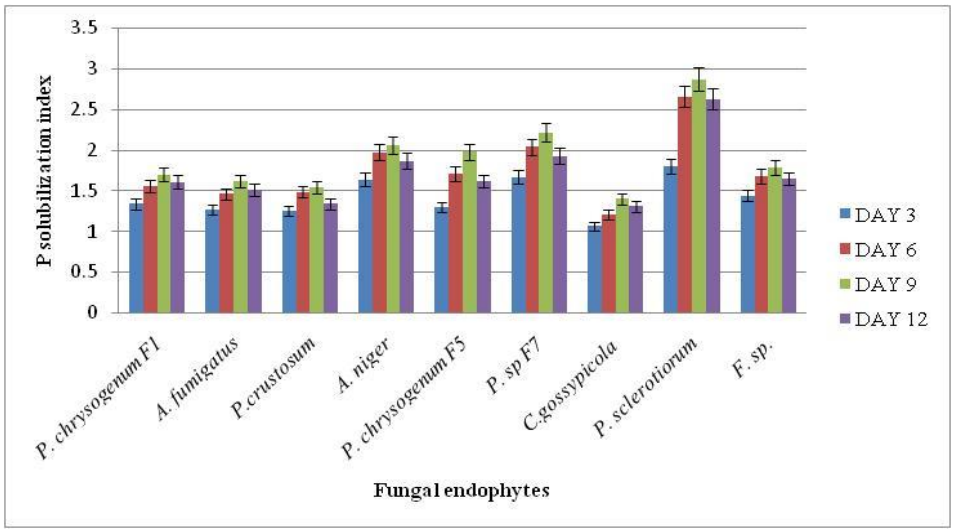

Fig. 1: Periodic Phosphate solubilization activity of endophytic fungi.

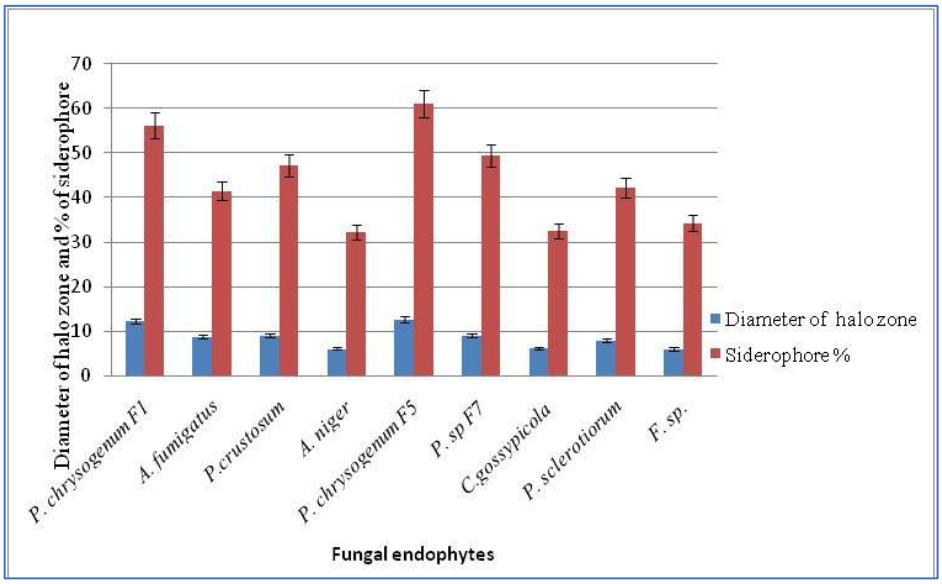

Fig. 2: Siderophore activity of endophytic fungi after 6 days of incubation

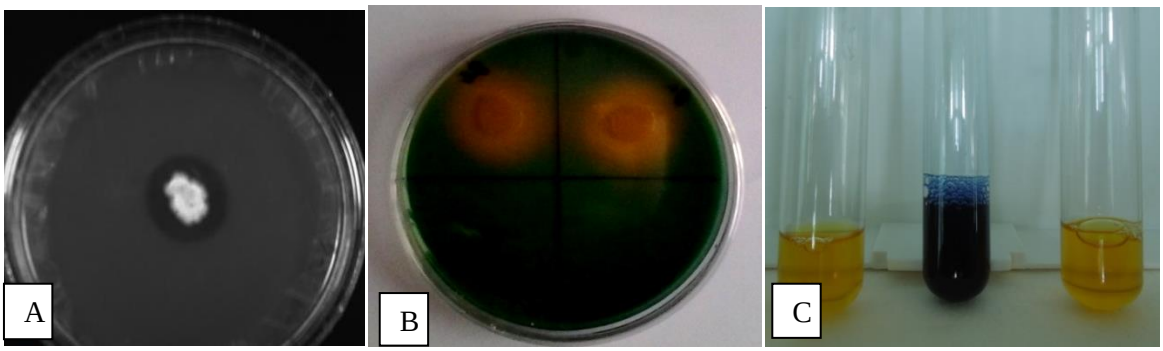

Fig. 3: (A) Phosphate solubilization activity, (B) Siderophore production in CAS agar plates and (C) Siderophore production activity in CAS shuttle assay by fungal endophytes of tea.

Extracellular enzyme production: The isolates were screened for their extra cellular enzyme producing activity using solid culture media. The halo zones around the colonies was considered positive and the diameter of the halo zones were recorded. Out of 9 fungal isolates, *A. niger* and *P. chrysogenum* F5 were found to be positive against all enzymes tested. Others were positive for only one, two or three enzymes. It was observed that all the isolates were positive for cellulase and pectinase production.

Quantitative estimation of extra cellular enzyme activity was measured in terms of amount of reducing sugar released to

per ml of supernatant per 30 minutes. The cellulase activity ranged between 20.20±2.6 (*P. sclerotiorum*) and 42.25±2.6 µg/ml (*P. chrysogenum* F5) of reducing sugar released per 30 minutes of incubation. The highest cellulase activity was recorded in *P. chrysogenum* F5 (42.25±2.6 µg/ml/30 min) followed by *C. gossypiicola* (38.2±2.1 µg/ml/30 min), *Penicillium* sp. F 7 (33.00±1.3 µg/ml/30 min) and *P. crustosum* (32.11±2.22 µg/ml/30 min). Only 4 isolates out of 9 were found to be amylase producers.

The highest amylase activity was found in *A. fumigatus* (41.50±1.54 µg/ml/30 min) followed by *A. niger* (37.00±2.22 µg/ml/30 min), *C. gossypiicola* (32.25±2.41 µg/ml/30 min) and *Penicillium* sp. F5 (31.22±1.76 µg/ml/30 min). Highest xylanase activity was observed in *A. niger* (32.00±2.18 µg/ml/30 min) followed by *P. crustosum* (28.00±2.32 µg/ml/30 min), *P. chrysogenum* F5(27.55±1.22 µg/ml/30 min) and the *Fusarium* sp. (25.25±1.23 µg/ml/30 min; Table 4; fig. 4).

Phylogenetic analysis of the endophytic fungi: Fungal isolates were characterized at molecular level by amplifying and sequencing their ITS region (Fig. 5). Identification of the endophytic fungi was done based on their similarity in BLAST. A phylogenetic tree was constructed to see their relationship. *Penicillium* was found to be the dominant group of endophytic fungi in tea bushes among the samples collected. The phylogenetic relationship among them is depicted in fig. 6.

Table 3
Siderophore activity of endophytic fungi.

Fungal endophytes	Diameter of the halo zone in mm	Siderophore % after Day 6
P. chrysogenum F1	12.33 ± 2.08	56.29 ± 0.43
A. fumigatus	8.81 ± 1.38	41.62 ± 1.25
P. crustosum	9.06 ± 1.92	47.27 ± 0.35
A. niger	6.21 ± 1.07	32.31 ± 0.7
***P. chrysogenum* F 5**	**12.78** ± 2.18	**61.09 ± 0.75**
Penicillium sp F 7	9.11 ± 1.08	49.48 ± 2.38
C. gossypicola	6.27 ± 1.17	32.58 ± 0.23
P. sclerotiorum	8.10 ± 1.73	42.28 ± 1.60
Fusarium sp.	6.10 ± 2.07	34.33 ± 0.55

Values are mean ± SD of five replicas

Table 4
Extracellular enzyme activity of the fungal endophytes of tea.

Fungal endophytes	Diameter of clear zones by isolates due to their enzyme activity. (All values are in mm.)				Amount of sugar released in µg/ml/30 min		
	Cellulase	Amylase	Pectinase	Xylanase	Cellulase	Amylase	Xylanase
P. chrysogenum F1	20 ± 2.1	-	11±1	-	26.50±2.3	-	-
A. fumigatus	20.25±2.3	24 ± 1.6	25.6±1.1	-	26.00±1.87	**41.50**±1.54	-
P. crustosum	24.5±2.0	-	36±2	18.6± .57	32.11±2.22	-	28.00±2.32
A. niger	23.5±2.6	20.25±1.2	38±2	21.3± 4.0	30.00±2.4	37.00±2.22	**32.00**±2.18
P. chrysogenum F5	29.5±1.94	18.25±17	29±1	16±1	**42.25**±2.6	31.22±1.76	27.55±1.22
Penicillium sp F 7	24.75±1.7	-	22.3±0.57	-	33.00±1.3	-	-
C. gossypicola	29.5±3.6	11.75±1.7	31.6±1.5	-	38.2±2.1	32.25±2.41	-
P. sclerotiorum	16.5±2.0	-	20±2.0	-	20.20±2.6	-	-
Fusarium sp.	20.75±2.5	-	19±1.0	15±1	25.55±1.72	-	25.25±1.23

Values are mean ± SD of five replicas

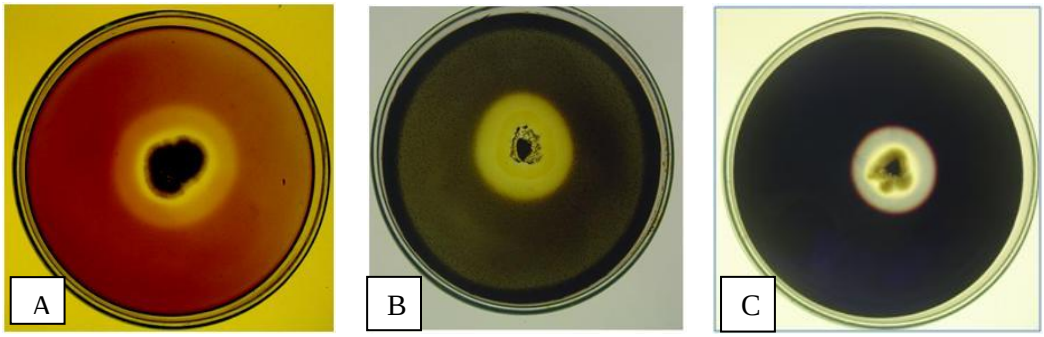

Fig. 4: (A) Production of cellulase, (B) Pectinase and (C) amylase by endophytic fungi of tea

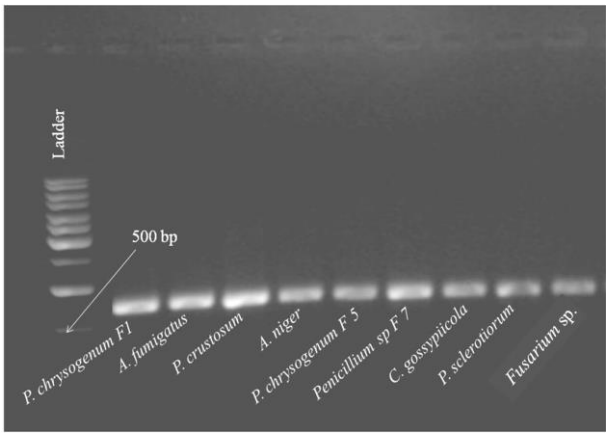

Fig. 5: PCR amplification of 18S ITS of the endophytic fungi of tea

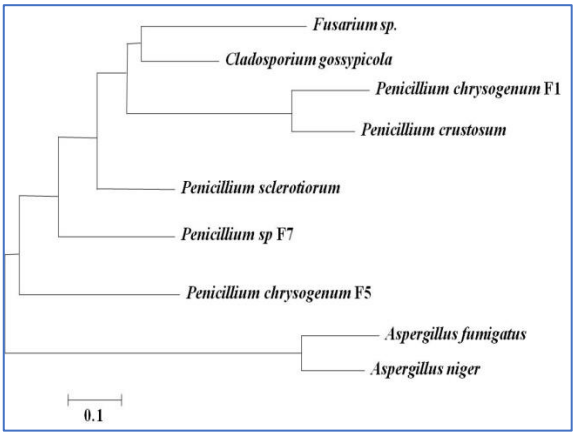

Fig. 6: Phylogenetic analysis of endophytic fungi of tea.

Discussion

It is now well established that the plant rhizosphere is one of the storehouses of plant growth-promoting microorganisms. From the rhizosphere, some of the microorganisms may enter into the plant's internal system through the cracks at the point of emergence of lateral roots, natural wounds and sometimes by secreting extracellular plant cell wall degrading enzymes and colonize in the root cortical region from where they may migrate to the aerial parts through the plant vascular system. Successful colonization by endophytes is affected by different factors like plant tissue type, the plant genotype, the microbial taxon and strain type and biotic and abiotic environmental conditions[11].

To escape from the plant defense mechanism, some endophytes secrete specific enzymes like superoxide dismutase, glutathione reductase (GR), catalases and hydroperoxide reductases in greater amounts that reduce the activity of ROS[12]. In our present study, among the root samples, the highest CFU was observed in the sample ORG-B (7.5×10^5) followed by ORG-A (7.4×10^5). Similarly, the fungal population in stem and leaf samples of ORG-A and ORG-B was significantly higher than in others. The lowest CFU in root, stem and leaf samples was observed in the samples collected from DOR. Both ORG-A and ORG-B samples were collected from organically maintained gardens.

However, sample DOR was collected from a tea garden near Digboi oil refinery which was agrochemical based. Meena et al[21] reported that most of the commonly used agrochemicals negatively affect soil microbiota and thereby causing an imbalance in soil health. Abosede[1] reported that oil pollution might affect soil's physical and biological properties. Pore spaces might be clogged which could reduce soil aeration and water infiltration and increase bulk density, subsequently affecting microbial growth. As the rhizosphere is the main source of endophytes, the effect of agrochemicals and oil contaminants might be a cause of depleted microbial population in the rhizosphere and internal to the plant. Out of 10 different fungi isolated from tea, 9 were phosphate solubilizers and siderophore producers. The highest phosphate solubilization was observed in *P. sclerotiorum* (2.87±0.01). Most of the *Penicillum* species isolated showed remarkable phosphate solubilization activity. Similarly, the siderophore production was highest in *P. chrysogenum* F5. Mehta et al[22] reported that the major endophytic P-solubilizing fungi belong to the genera *Penicillium*, *Aspergillus*, *Piriformospora* and *Curvularia*; and are more competitive and aggressive colonizers than non-endophytic microbes.

Apart from mineral solubilization, endophytic fungi are good siderophore producers and hence can help the host in iron uptake. Srinivas et al[35] isolated endophytic fungi from different plant species and found that species of *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium* etc. have very high siderophore producing activity and were reported for their ability to promote plant growth.

All the fungal isolates were found positive for cellulase and pectinase production. Others were positive for one, two, or three enzymes only. Most of the fungal endophytes reported are more or less good sources of extracellular enzymes. They secrete these enzymes to obtain their nutrition from the host as well as a resistance mechanism against pathogenic invasion[39]. Endophytic fungi like *Cladosporium*, *Rhizoctonia*, *Aspergillus*, *Chaetomium*, *Biosporus*, *Fuzarium*, *Curvularia*, *Penicillium*, *Fusarium*, *Cladosporium* and *Colletotrichum* are very good sources of extracellular enzynes[19,20,25,37].

The results of the present study supported by the works of earlier workers reveal that endophytic fungi of tea (*Camellia sinensis*) are not only a good source of plant growth but also a good source of extracellular enzymes. Proper identification and bioprospecting of these endophytic fungal complexes of *Camellia sinensis* may lead to the inventorization of fungi with high bio fertilizer and other natural products useful for mankind.

Conclusion

In the present study, the endophytic fungi isolated from tea leaf, stem and root showed remarkable phosphate

solubilization, siderophore production and extracellular enzyme producing activities. Proper documentation and bio-prospecting may lead to identification of endophytes with remarkable plant growth promoting and enzyme producing activities.

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