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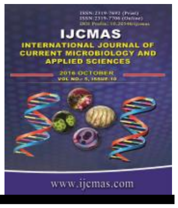

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Isolation and Identification of Some Dark Septate Endophytic Fungi (DSE) from the Roots of the Ethno-Medicinal Plant *Plumbago zeylanica* L.

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ABSTRACT

Keywords

Dark septate endophytic fungi; *Perenniporia* sp.; *Aspergillus niger; Plumbago zeylanica* L.

Article Info

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In present study an attempt has been made to isolate and identify the dark septate endophytic fungi (DSE) and their colonization in the roots of an important ethno-medicinal plant, *Plumbago zeylanica* L. The plant exhibited significant colonization by DSE. Two different strains of dark septate endophytic fungi were isolated from the roots of the plant. The pure cultures were established (PUB and PUD). DNA was isolated from the Pure culture – PUB and PUD and 28s rDNA sequencing of D_1, D_2 region of the larger sub unit was performed. Sequence analysis exhibited sequence similarity with *Perenniporia* sp. CBS 130020 (Gen Bank Accession Number: JX292098.1) and *Aspergillus niger* strain RA402 (GenBank Accession Number: GQ169753.1) respectively based on nucleotide homology and phylogenetic analysis.

Introduction

Vascular plants host a great variety of fungi. In addition to being susceptible to soil-borne pathogens, plant roots are also colonized by non-pathogenic or mutualistic fungi like arbuscular mycorrhizae (AM), ectomycorrhizae (EM) and dark septate endophytic fungi (DSE). A vast majority of terrestrial plant species form mycorrhizal associations (Harley and Smith, 1983, Smith and Read, 1997). It is now evident that the mycorrhizal fungi have many significant functions in ecosystem like they absorb non-mobile nutrients from the soil and translocate them to host plants, sequester potentially harmful heavy metal ions, facilitate interplant transfer of nutrients, and beneficially modify plant water relations (Smith and Read, 1997).

In contrast to the surfeit of knowledge about the EM and AM fungi, very little is known about the DSE. The DSE are broadly classified as conidial or sterile septate fungal endophytes that form melanized structures such as inter- and intracellular hyphae and microsclerotia within the plant roots and that have known or likely affinities with ascomycetes (Jumpponen and Trappe, 1998).

DSE are found worldwide and coexist often with different mycorrhizal fungi. They have been reported from many plant species including plants that have been considered non-mycorrhizal (Jumpponen and Trappe, 1998).

DSE fungi under some environmental or experimental conditions, can enhance host growth and nutrient uptake, hence functioning in a manner typical of mycorrhizal associations. Enhanced growth and improved nutritional status indicate better performance resulting from DSE colonization. It has been suggested that the microsclerotia may serve as dispersal structures (Currah and Tsuneda, 1993), although the actual purpose appears to be unknown. Perhaps they serve a storage function for the fungi as do the vesicles of AM fungi.

There are three different mechanisms through which DSE fungi may inhibit pathogens, or minimize their impact on plant growth and performance. Firstly, mycorrhizal fungi and rhizosphere-inhabiting pathogens may compete for the same recourse of plant photosynthates or for sites of colonization. Secondly, compounds inhibitory to pathogens may be produced. Finally, the DSE colonization may have prophylactic value by inducing plant defense responses to subsequent pathogen infection (Mandyam and Jumpponen, 2005). Some strains of DSE may be involved in host plant nutrient acquisition and it has been proposed that this may be a mutualistic, mycorrhiza-like relationship which do not appear to be host specific (Jumpponen and Trappe, 1998),

Research works on DSE colonization in various groups of plants is still limited. Hence, in present investigation, our endeavor has impelled us to isolate and identify the DSE form the roots of *Plumbago zeylanica* L. an ethno medicinally important herb having strong female abortificant property.

Materials and Methods

Secondary and tertiary roots were collected from the plant *Plumbago zeylanica* L., Freshly collected roots were rinsed in distilled water for several times to remove all the soil debris. Surface sterilization of the roots was done with ethyl alcohol, 0.01% NaOCl and antibiotic water successively to disinfect the roots and to kill the bacteria and fungal spores remaining outside.

Roots were cut opened in Y shape with the help of a sterilized blade, placed in sterilized PDA media supplemented with antibiotic to prevent any Bacterial growth.

After observing fungal growth, pure culture was established by serial dilution method.

DNA was isolated from the Pure culture – PUB and PUD. Quality was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA was observed.

Fragment of D2 region of *28S rDNA* was amplified by PCR from the above isolated genomic DNA. A single discrete PCR amplicon band of 700bp was observed when resolved on Agarose Gel (Figure 1a,b respectively)

The PCR amplicon was purified and further processed for the sequencing.

Forward and Reverse DNA sequencing reaction of PCR amplicon was carried out with DF and DR indicator and (DF:ACCCGCTGAACTTAAGC, DR: GGT CCG TGT TTC AAG ACG G) primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

Consensus sequence of 641 bp of D2 region of LSU gene was generated from forward and reverse sequence data using aligner software.

The D2 region of *28S rDNA* sequence was used to carry out BLAST with the nr-database of NCBI genbank database. Based on maximum identity score, fifteen sequences were selected and the phylogenetic tree was constructed using MEGA 5.

The evolutionary history was inferred using the Neighbor-Joining method [Felsenstein, 1985]. The bootstrap consensus tree inferred from 1000 replicates [Harley et al., 1983] is taken to represent the evolutionary history of the taxa analyzed [Harley et al., 1983].

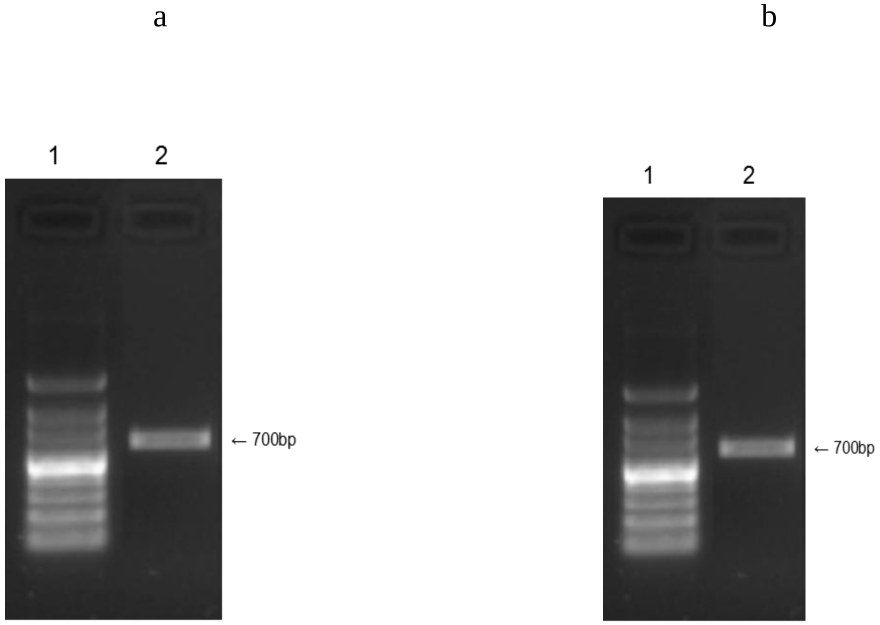
Results and Discussion

The culture, labeled as PUB exhibited sequence similarity to *Perenniporia* sp. CBS 130020 GenBank Accession Number: JX292098.1) and the culture, PUD to *Aspergillus niger* strain RA402 (GenBank Accession Number: GQ169753.1) based on nucleotide homology and phylogenetic analysis.

Information about other close homologs for the microbes can be found from the Alignment View table and from the phylogenetic tree.

Fig.1 1.0% Agarose gel showing 700bp amplicon (D2 region) of 28S rDNA

Quality Check on 1.0% Agarose gel



Lane 1: 1 Kb DNA Ladder
Lane 2: 700bp amplicon (D2 region) of *28S rDNA*

Sequence data of the sample PUB

1. PUB-2B_DF_S014381_A05_047.ab1: Data obtained with Forward primer
2. PUB-2B_DR_S014381_B05_045.ab1: Data obtained with Reverse primer
PUB-2B_DF_S014381_A05_047 (565 bp)

GCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGG
ACCGTGTATAAGTCTCTTGGAACAGAGCGTCGTAGAGGGTGAGAATCCCGTCTTTGACACGGACTACC
AGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTAAATT
CCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGC
ACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCT
GCCGGAACTCAGCCTTGCTTTTGCTTGGTGCACTTTCCGGTTGACGGGCCAGCATCGATTTTGACCGTC
GGAAAAGGGCTGGAGAAATGTGGCACCTCCGGGTGTGTTATAGTCTTCAGTCGTATACGGCGGTTGGG
ATCGAGGAACGCAGCGCGCCGCAAGGCAGGGGTTCGCCCACTATCGCGCTTAGGATGCTGGCGTAAT
GGCTTTAAACGACCCGTCTTG

PUB-2B_DR_S014381_B05_045 (602 bp)

GCGCGATAGTGGGCGAACCCCTGCCTTGCGGCGCGCTGCGTTCCTCGATCCCAACCGCCGTATACGAC
TGAAGACTATAACACACCCGGAGGTGCCACATTTCTCCAGCCCTTTTCCGACGGTCAAAATCGATGCT
GGCCCGTCAACCGGAAAGTGCACCAAGCAAAAGCAAGGCTGAGTTCCGGCAGACGCGACTGACTTCA
AGCGTTTCCCTTTCAGCAATTTCACGTACTGTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTCCCTCA
CGGTACTTGTTCGCTATCGGTCTCTCGCCAATATTTAGCTTTAGATGGAATTTACCACCCATTTTGAGCT
GCATTCCCAAACAACTCGACTCTTTGAGAGCGCATCACAAAGCACTGGTAGTCCGTGTCAAAGACGGG
ATTCTCACCCTCTACGACGCTCTGTTCCAAGAGACTTATACACGGTCCAGCGCGGAAAGCACTTCTCCA
GACTACAACTCGGACGGCCAAAGACCGCCAGATTTTAAATTTGAGCTTTTCCCGCTTCACTCGCAGTTA
CTAGGGGAATCCTTGTTAGTTTCTTTTCCTCCGCTTATTGATATGCTTAATAA

Consensus Sequence PUB (641 bp)

TTATTAAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACTGCGAGTGAAGC
GGGAAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCC
GCGCTGGACCGTGTATAAGTCTCTTGGAACAGAGCGTCGTAGAGGGTGAGAATCCCGTCTTTGACACGG
ACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGT
AAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAA
AAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGC
GTCTGCCGGAACTCAGCCTTGCTTTTGCTTGGTGCACTTTCCGGTTGACGGGCCAGCATCGATTTTGACC
GTCGGAAAAGGGCTGGAGAAATGTGGCACCTCCGGGTGTGTTATAGTCTTCAGTCGTATACGGCGGTTG
GGATCGAGGAACGCAGCGCGCCGCAAGGCAGGGGTTCGCCCACTATCGCGCTTAGGATGCTGGCGTAA
TGGCTTTAAACGACCCGTCTTG

Sequence data of the sample PUD

1. PUD-2H_DF_S014381_G04_020.ab1: Data obtained with Forward primer
2. PUD-2H_DR_S014381_H04_018.ab1: Data obtained with Reverse primer
PUD-2H_DF_S014381_G04_020 (520 bp)

GCTGGCTCCTTCGGAGTCCGCATTGTAATTTGCAGAGGATGCTTTGGGTGCGGCCCCCGTCTAAGTGCC
CTGGAACGGGCCGTCAGAGAGGGTGAGAATCCCGTCTTGGGCGGGGTGTCCGTGCCCGTGTAAAGCTC
CTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTTCATCTAAAGCTAAATA
CTGGCCGGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGCACTTTGAAAAGAGAGTT
AAACAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGACCAGACTCGCCCGCGGGGTTCAGCCGGC
ATTCGTGCCGGTGTACTTCCCCGTGGGCGGGCCAGCGTCGGTTTGGGCGGCCGGTCAAAGGCCCCTGG
AATGTAGTGCCCTCCGGGGCACCTTATAGCCAGGGGTGCAATGCGGCCAGCCTGGACCGAGGAACGC
GCTTCGGCACGGACGCTGGCATAATGGTCGTAAACGACCCGTCTTG

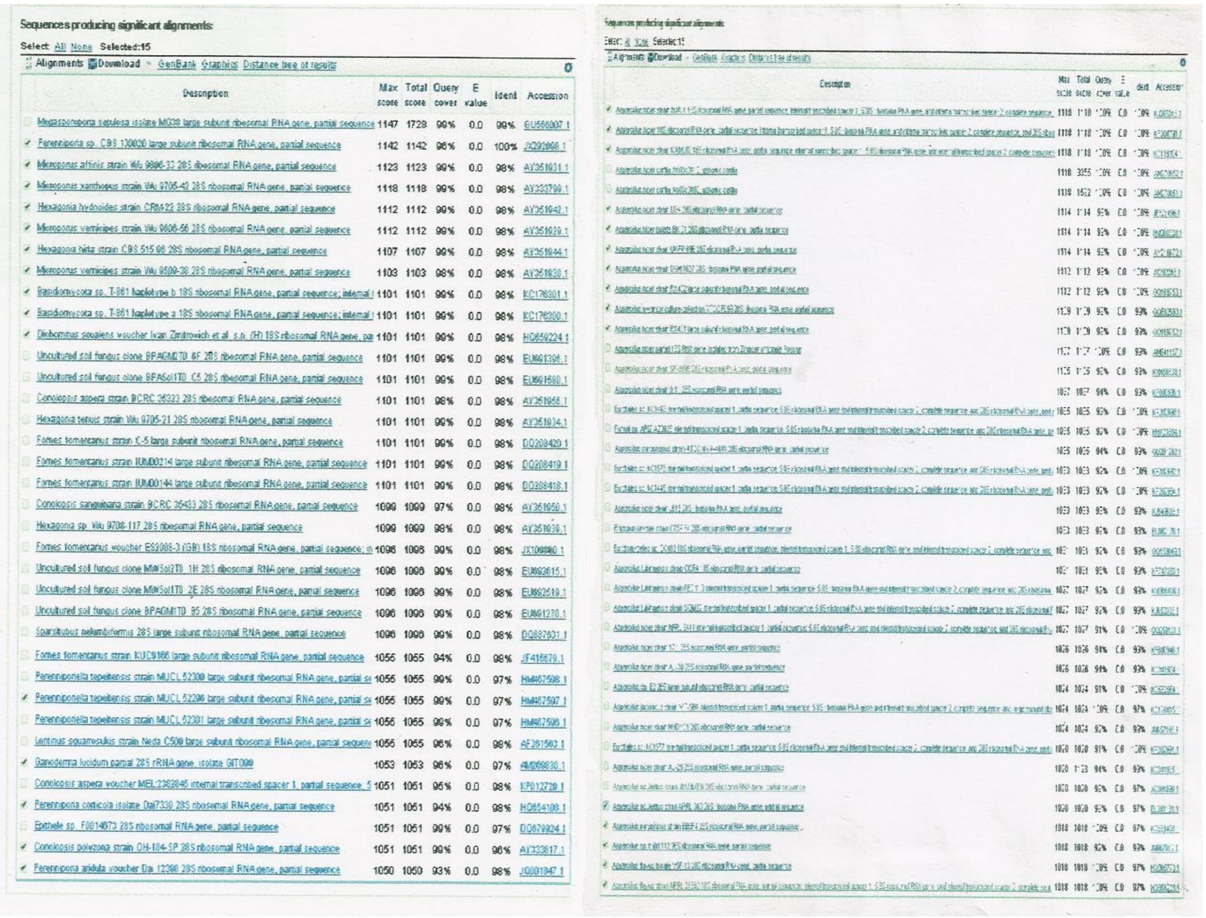
PUD-2H_DR_S014381_H04_018 (544 bp)

GGCTGGCCGCATTGCACCCCTGGCTATAAGGTGCCCCGGAGGGCACTACATTCCAGGGGCCTTTGACCG
GCCGCCCAAACCGACGCTGGCCCGCCCACGGGGAAGTACACCGGCACGAATGCCGGCTGAACCCCGC
GGGCGAGTCTGGTCGCAAGCGCTTCCCTTTCAACAATTTCACGTGCTGTTTAACTCTCTTTTCAAAGTGC
TTTTCATCTTTCGATCACTCTACTTGTGCGCTATCGGTCTCCGGCCAGTATTTAGCTTTAGATGAAATTTAC
CACCCATTTAGAGCTGCATTCCCAAACAACTCGACTCGTCGAAGGAGCTTTACACGGGCACGGACACCC
CGCCCAAGACGGGATTCTCACCCTCTCTGACGGCCCGTTCCAGGGCACTTAGACGGGGGCCGCACCCA
AAGCATCCTCTGCAAATTACAATGCGGACTCCGAAGGAGCCAGCTTTCAAATTTGAGCTCTTGCCGCTT
CACTCGCCGTTACTGAGGCAATCCCGGTTGGTTTCTTTTCCTCCGCTTATTGATATGCTT

Consensus Sequence PUD (632 bp)

TTTGGGTCCGTTGTTCAAGACGGGCGGATTACGACCATTACGCCAGCATCCTAGCCGAAGCGCGGACCT
CGCCCGGCGCTCGCTGCATCACAAGAGGCTATAACACTCCCGAGGGAGCTACATTCCTCAAGTCTTATC
CAGCGGCACCAGACGATGCTGGCCTGCAACGCGGCGAGTAGACCGGTCAGAAGACCGGCGGAACACC
GCGAGCAAGTCTGGTTGCAATCCCTTCCCTTTTAACAATTTCACGTGCTTTTTAACTCTCTTTCCAAAGT
GCTTTTCATCTTTCGATCACTCTACTTGTGCGCTATCGGTCTCTGGCCAATATTTAGCTTTAGAAGAAATTT
ACCTCCCATTTAGAGCTGCATTCCCAAACAACTCGACTCGTCGAAGGAGCTACGTATAGAGCACCTTTC
CGACCGCATACGGGATTCTCACCCTCTATGACGTCCTGTTCCAAGGAACTTAGGTCGGTGGTTACTCAGA
AGCATCCTCTACAAATTACAACTCGGACGTCGAAGACGCCAGATTTCAAATTTGAGCTATTGCTGCTTCA
CTCGCCGTTACTAGAGCAATCCCTGTTGGTTTCTTTTCCTCCGCTTATTGATATGCTTAAGTCCCACCGGG
GTAAA

Distribution of 101 Blast Hits on the Query Sequence



Sequences producing significant alignments:

Select: All None Selected:15

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Megasporoporia setulosa isolate MG38 large subunit ribosomal RNA gene, partial sequence	1147	1728	99%	0.0	99%	GU566007.1
☑	Perenniporia sp. CBS 130020 large subunit ribosomal RNA gene, partial sequence	1142	1142	96%	0.0	100%	JX290068.1
☑	Microporus affinis strain Wu 9806-33 28S ribosomal RNA gene, partial sequence	1123	1123	99%	0.0	98%	AY351931.1
☑	Microporus xanthopus strain Wu 9706-42 28S ribosomal RNA gene, partial sequence	1118	1118	99%	0.0	98%	AY333799.1
☑	Hexagonia hydnoides strain CRM-22 28S ribosomal RNA gene, partial sequence	1112	1112	99%	0.0	98%	AY351942.1
☑	Microporus vernicipes strain Wu 9606-56 28S ribosomal RNA gene, partial sequence	1112	1112	99%	0.0	98%	AY351929.1
☑	Hexagonia hirta strain CBS 515.96 28S ribosomal RNA gene, partial sequence	1107	1107	99%	0.0	98%	AY351944.1
☑	Microporus vernicipes strain Wu 9509-38 28S ribosomal RNA gene, partial sequence	1103	1103	98%	0.0	98%	AY351930.1
☑	Basidiomycota sp. T-861 haplotype b 18S ribosomal RNA gene, partial sequence; internal	1101	1101	99%	0.0	98%	KC176301.1
☑	Basidiomycota sp. T-861 haplotype a 18S ribosomal RNA gene, partial sequence; internal	1101	1101	99%	0.0	98%	KC176300.1
☑	Dichomitus squalens voucher Ivan Zmitrovich et al. s.n. (H) 18S ribosomal RNA gene, pa	1101	1101	99%	0.0	98%	HQ659224.1
☐	Uncultured soil fungus clone BPAGM2T0_6F 28S ribosomal RNA gene, partial sequence	1101	1101	99%	0.0	98%	EU881396.1
☐	Uncultured soil fungus clone BPASoil1T0_C5 28S ribosomal RNA gene, partial sequence	1101	1101	99%	0.0	98%	EU881680.1
☐	Coniolopsis aspera strain BCRC 36323 28S ribosomal RNA gene, partial sequence	1101	1101	98%	0.0	98%	AY351968.1
☐	Hexagonia tenuis strain Wu 9705-21 28S ribosomal RNA gene, partial sequence	1101	1101	99%	0.0	98%	AY351934.1
☐	Fomes fomentarius strain C-5 large subunit ribosomal RNA gene, partial sequence	1101	1101	99%	0.0	98%	DQ208420.1
☐	Fomes fomentarius strain IUM0214 large subunit ribosomal RNA gene, partial sequence	1101	1101	99%	0.0	98%	DQ208419.1
☐	Fomes fomentarius strain IUM0144 large subunit ribosomal RNA gene, partial sequence	1101	1101	99%	0.0	98%	DQ208418.1
☐	Coniolopsis sanguinaria strain BCRC 36433 28S ribosomal RNA gene, partial sequence	1099	1099	97%	0.0	98%	AY351950.1
☐	Hexagonia sp. Wu 9708-117 28S ribosomal RNA gene, partial sequence	1099	1099	98%	0.0	98%	AY351939.1
☐	Fomes fomentarius voucher ES2008-3 (GB) 18S ribosomal RNA gene, partial sequence; in	1096	1096	99%	0.0	98%	JX109860.1
☐	Uncultured soil fungus clone MWSoil2T0_1H 28S ribosomal RNA gene, partial sequence	1096	1096	99%	0.0	98%	EU882615.1
☐	Uncultured soil fungus clone MWSoil1T0_2E 28S ribosomal RNA gene, partial sequence	1096	1096	99%	0.0	98%	EU882519.1
☐	Uncultured soil fungus clone BPAGM1T0_95 28S ribosomal RNA gene, partial sequence	1096	1096	99%	0.0	98%	EU881270.1
☐	Sparsitubus nelumbiformis 28S large subunit ribosomal RNA gene, partial sequence	1096	1096	99%	0.0	98%	DQ887631.1
☐	Fomes fomentarius strain KUC9168 large subunit ribosomal RNA gene, partial sequence	1055	1055	94%	0.0	98%	JF416679.1
☐	Perenniporiella tepeitensis strain MUCL 52300 large subunit ribosomal RNA gene, partial se	1055	1055	99%	0.0	97%	HM467598.1
☑	Perenniporiella tepeitensis strain MUCL 52296 large subunit ribosomal RNA gene, partial se	1055	1055	99%	0.0	97%	HM467597.1
☐	Perenniporiella tepeitensis strain MUCL 52301 large subunit ribosomal RNA gene, partial se	1055	1055	99%	0.0	97%	HM467596.1
☐	Lentinus squarrosulus strain Neda C500 large subunit ribosomal RNA gene, partial sequen	1055	1055	96%	0.0	98%	AF261563.1
☑	Ganoderma lucidum partial 28S rRNA gene, isolate GIT008	1053	1053	96%	0.0	97%	AM269830.1
☐	Coniolopsis aspera voucher MEL:2382845 internal transcribed spacer 1, partial sequence; 5	1051	1051	95%	0.0	98%	KP012729.1
☑	Perenniporia corticola isolate Dai7330 28S ribosomal RNA gene, partial sequence	1051	1051	94%	0.0	98%	HQ654108.1
☐	Echinodontium sp. F0014673 28S ribosomal RNA gene, partial sequence	1051	1051	99%	0.0	97%	DQ679924.1
☑	Coriolopsis polyzona strain OH-184-SP 28S ribosomal RNA gene, partial sequence	1051	1051	99%	0.0	96%	AY333817.1
☑	Perenniporia aridula voucher Dai 12398 28S ribosomal RNA gene, partial sequence	1050	1050	93%	0.0	98%	JQ001847.1

a b

BLAST DATA of PUB and PUD (Alignment view using combination of NCBI GenBank)

Significant Alignments Produced by the Sequence:

1. **PUB**

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
JX292098.1	*Perenniporia* sp. CBS 130020	1142	1142	96%	0.0	100%
AY351931.1	*Microporus affinis* strain Wu 9806-33	1123	1123	99%	0.0	98%
AY333799.1	*Microporus xanthopus* strain Wu 9705-42	1118	1118	99%	0.0	98%
AY351942.1	*Hexagonia hydnoides* strain CRM-22	1112	1112	99%	0.0	98%
Y351929.1	*Microporus vernicipes* strain Wu 9606-56	1112	1112	99%	0.0	98%
AY351944.1	*Hexagonia hirta* strain CBS 515.96	1107	1107	99%	0.0	98%
AY351930.1	*Microporus vernicipes* strain Wu 9509-38	1103	1103	98%	0.0	98%
KC176301.1	*Basidiomycota* sp. T-861 haplotype b	1101	1101	99%	0.0	98%
KC176300.1	*Basidiomycota* sp. T-861 haplotype a	1101	1101	99%	0.0	98%
HQ659224.1	*Dichomitus squalens* voucher	1101	1101	99%	0.0	98%
HM467597.1	*Perenniporiella tepeitensis* strain MUCL 52296	1055	1055	99%	0.0	97%
AM269830.1	*Ganoderma lucidum* isolate GlTO99	1053	1053	96%	0.0	97%
HQ654108.1	*Perenniporia corticola* isolate Dai7330	1051	1051	94%	0.0	98%
AY333817.1	*Coriolopsis polyzona* strain OH-184-SP	1051	1051	99%	0.0	96%
JQ001847.1	*Perenniporia aridula* voucher Dai 12398	1050	1050	93%	0.0	98%

2. **PUD**

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
KJ365316.1	*Aspergillus niger* strain NJA-1	1118	1118	100%	0.0	100%
KF304798.1	*Aspergillus niger*	1118	1118	100%	0.0	100%
KC119204.1	*Aspergillus niger* strain KAML02	1118	1118	100%	0.0	100%
JF521496.1	*Aspergillus niger* strain US4	1114	1114	99%	0.0	100%
HM008328.1	*Aspergillus niger* isolate BK 01	1114	1114	99%	0.0	100%
AY216672.1	*Aspergillus niger* strain UWFP 696	1114	1114	99%	0.0	100%

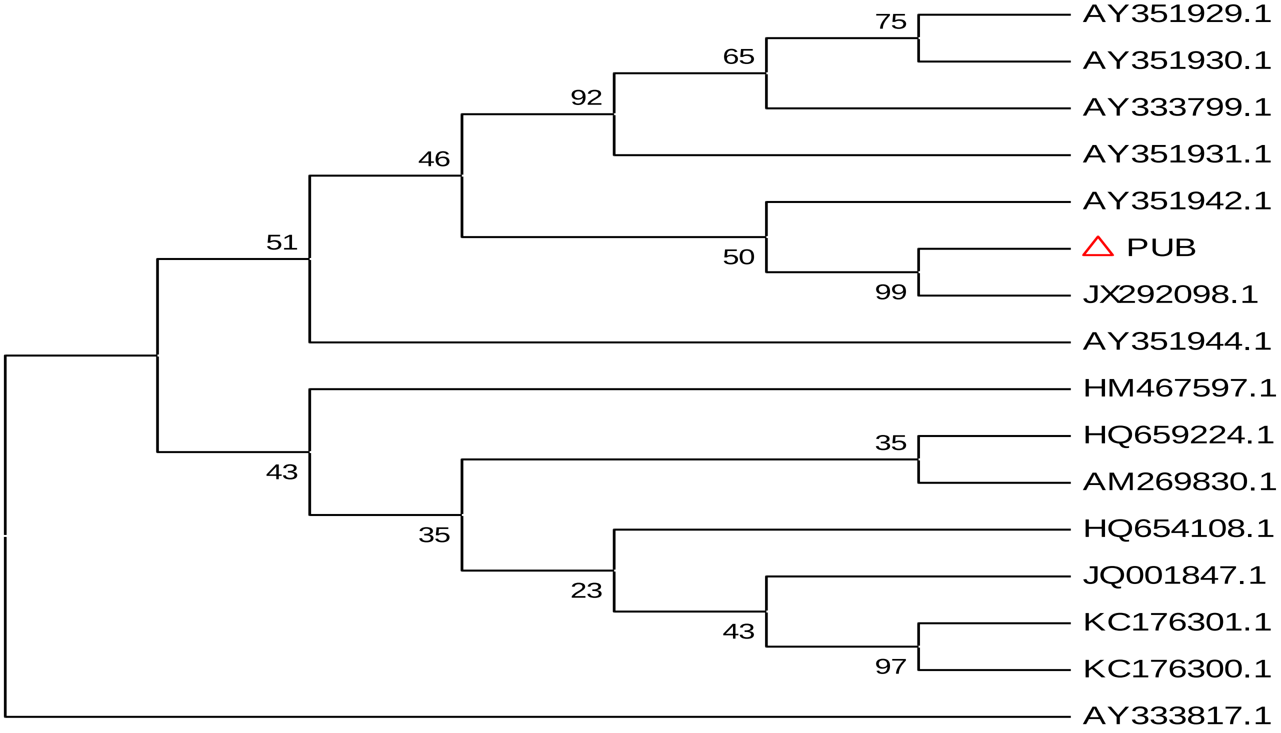
Accession	Description	Max score	Total score	Query coverage	E value	Max ident
JX292268.1	*Aspergillus niger* strain DSM 1627	1112	1112	99%	0.0	100%
GQ169753.1	*Aspergillus niger* strain RA402	1112	1112	99%	0.0	100%
GQ892590.1	*Aspergillus awamori* culture-collection VTCC:F099	1109	1109	99%	0.0	99%
GQ169752.1	*Aspergillus niger* strain RA401	1109	1109	99%	0.0	99%
EU381181.1	*Aspergillus aculeatus* strain NRRL 360	1020	1020	99%	0.0	97%
KC589438.	*Aspergillus parasiticus* strain BBEF4	1018	1018	100%	0.0	97%
JN967012.1	*Aspergillus* sp. trijbl1112	1018	1018	92%	0.0	97%
HQ395773.1	*Aspergillus flavus* isolate V5F-13	1018	1018	100%	0.0	97%
HQ856223.1	*Aspergillus flavus* strain NRRL 21882	1018	1018	100%	0.0	97%

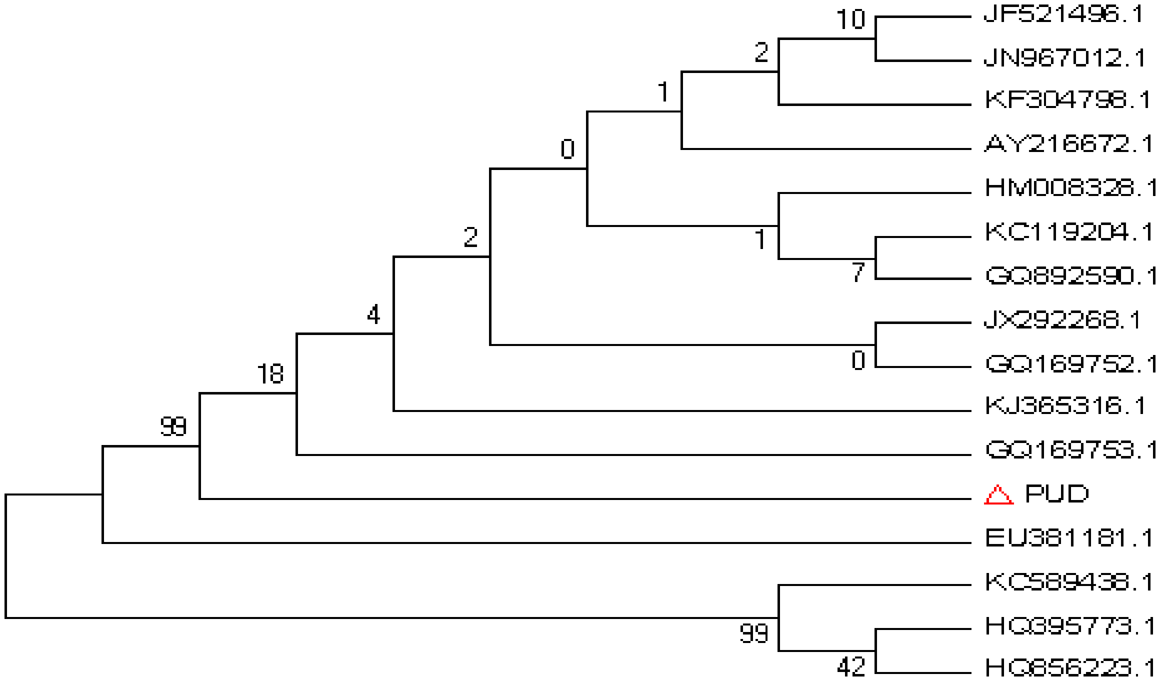
Distance Matrix of the sample PUB and PUD

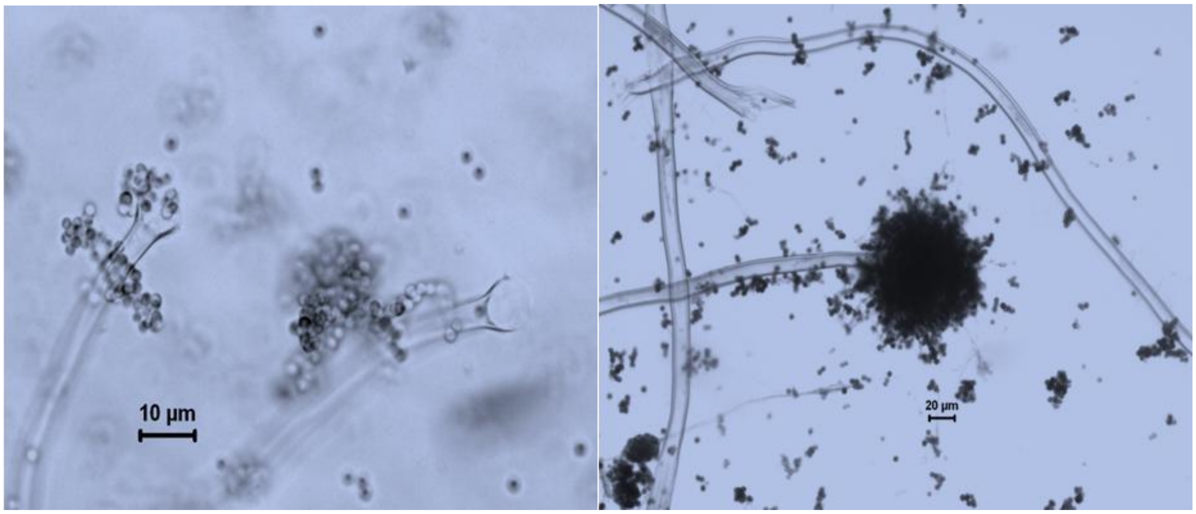
		1	2	3	4	5	6	7	8	9	10	11
PUB	1		0.0000	0.0053	0.0057	0.0058	0.0061	0.0061	0.0061	0.0063	0.0063	0.0061
JX292098.1	2	0.0000		0.0053	0.0057	0.0058	0.0061	0.0061	0.0061	0.0063	0.0063	0.0061
AY351931.1	3	0.0170	0.0170		0.0016	0.0048	0.0024	0.0042	0.0024	0.0047	0.0047	0.0052
AY333799.1	4	0.0188	0.0188	0.0017		0.0051	0.0016	0.0046	0.0016	0.0050	0.0050	0.0056
AY351942.1	5	0.0205	0.0205	0.0136	0.0153		0.0055	0.0061	0.0055	0.0065	0.0065	0.0056
Y351929.1	6	0.0205	0.0205	0.0034	0.0017	0.0170		0.0050	0.0000	0.0053	0.0053	0.0058
AY351944.1	7	0.0222	0.0222	0.0118	0.0135	0.0222	0.0153		0.0050	0.0053	0.0053	0.0054
AY351930.1	8	0.0205	0.0205	0.0034	0.0017	0.0170	0.0000	0.0153		0.0053	0.0053	0.0058
KC176301.1	9	0.0239	0.0239	0.0135	0.0153	0.0240	0.0170	0.0187	0.0170		0.0000	0.0048
KC176300.1	10	0.0239	0.0239	0.0135	0.0153	0.0240	0.0170	0.0187	0.0170	0.0000		0.0048
HQ659224.1	11	0.0239	0.0239	0.0170	0.0187	0.0204	0.0204	0.0187	0.0204	0.0136	0.0136	

		1	2	3	4	5	6	7	8	9	10	11
PUD	1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000	0.0000
KJ365316.1	2	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000
KF304798.1	3	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000
KC119204.1	4	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0000	0.0017	0.0000
JF521496.1	5	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0000	0.0017	0.0000
HM008328.1	6	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0000	0.0017	0.0000
AY216672.1	7	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0000	0.0017	0.0000
JX292268.1	8	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0000	0.0017	0.0000
GQ169753.1	9	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.0017	0.0000
GQ892590.1	10	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018	0.0018		0.0017
GQ169752.1	11	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0018	

Phylogenetic Tree:







PUB **PUD**

Microscopic view of the samples

Identification of fungi using conventional methods like growth characteristics, morphology, colony pattern, and biochemical reactions may be difficult. DNA sequencing of ribosomal RNA genes is becoming the most appropriate alternative to the conventional methods for identifying fungi. The ribosomal RNA genes exhibit markedly variable sequences when comparing between different species. These genes also have highly conserved sequences between all fungi, and these regions are used as primer binding sites for amplification and sequencing reactions. Fungal genomic DNA is extracted from a pure culture and amplified using PCR reaction. Sequencing results are used to search databases of verified fungal sequences and the percent match to the top hits is calculated and evaluated along with phylogenetic trees of relatedness. These results are compared to the traditional methods used in identification of the organisms. In present investigation, the culture, labeled as PUB exhibited similarity to *Perenniporia* sp. CBS 130020 GenBank Accession Number: JX292098.1) and the culture, PUD to *Aspergillus niger* strain RA402 (GenBank Accession Number: GQ169753.1) based on nucleotide homology and phylogenetic analysis.

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