Diversity and bioactivities of fungal endophytes from *Distylium chinense*, a rare waterlogging tolerant plant endemic to the Three Gorges Reservoir

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**Abstract**

**Background:** The present study involves diversity and biological activities of the endophytic fungal community from *Distylium chinense*, a rare waterlogging tolerant plant endemic to the Three Gorges Reservoir. This study has been conducted hypothesizing that the microbial communities in the TGR area would contribute to the host plant tolerating a range of abiotic stress such as summer flooding, infertility, drought, salinity and soil erosion etc., and they may produce new metabolites, which may possess plentiful bioactive property, especially antioxidant activity. Therefore in the current study, the antioxidant, antimicrobial and anticancer activities of 154 endophytes recovered from *D. chinense* have been investigated. Furthermore, the active metabolites of the most broad-spectrum bioactive strain have also been studied.

**Results:** A total of 154 fungal endophytes were isolated from roots and stems. They were categorized into 30 morphotypes based on cultural characteristics and were affiliated with 27 different taxa. Among these, the most abundant fungal orders included Diaporthales (34.4%) and Botryosphaeriales (30.5%), which were predominantly represented by the species *Phomopsis* sp. (24.7%) and *Neofusicoccum parvum* (23.4%). Fermentation extracts were evaluated, screening for antioxidant, antimicrobial and anticancer activities. Among the 154 isolates tested, 99 (64.3%) displayed significant antioxidant activity, 153 (99.4%) exhibited inclusive antimicrobial activity against at least one tested microorganism and 27 (17.5%) showed exclusive anticancer activity against one or more cancer cell lines. Specifically, the crude extract of *Irpex lacteus* DR10–1 exhibited note-worthy bioactivities. Further chemical investigation on DR10–1 strain resulted in the isolation and identification of two known bioactive metabolites, indole-3-carboxylic acid (1) and indole-3-carboxaldehyde (2), indicating their potential roles in plant growth promotion and human medicinal value.

**Conclusion:** These results indicated that diverse endophytic fungal population inhabits *D. chinense*. One of the fungal isolate DR10–1 (*Irpex lacteus*) exhibited significant antioxidant, antimicrobial and anticancer potential. Further, its active secondary metabolites 1 and 2 also showed antioxidant, antimicrobial and anticancer potential.

**Keywords:** *Distylium chinense*, Bioactivity, Endophytic fungi, Identification, Metabolites

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Background

Endophytic fungi in plants are microorganisms that parasitize symbiotically in the internal tissues during the whole or part of their life cycles of the hosts without causing apparent pathogenic symptoms [1], but may turn pathogenic during host senescence [2]. Accumulated evidence has confirmed that plant endophytes from special or extreme environment has many effects on host ecological adaptability [3–5]. It is well known that the concurrence of endophytes may accelerate plant growth and increase the survival rate of biotic or abiotic stresses, such as plant diseases, pests, drought, salinity and extreme temperatures [6–9]. Specifically, some endophytes are beneficial to plants by producing special substances, such as secondary metabolites, which can prevent the host from being attacked successfully by fungi and pests [10]. So far, endophytes, especially those under complex and extreme conditions, have been shown to produce a variety of metabolites with complex structures, such as alkaloids, terpenoids, polyketides, lipids, glycosides, isoprenoids, and hybrids of those metabolites, etc. [11–13]. More interestingly, these metabolites also showed a variety of interesting bioactivities including antifungal [14], antibacterial [15], anticancer [16], anti-HIV [17], antioxidants [18], etc. Due to these, endophytes from an untapped diverse habitat are a significant source of novel and natural drugs [19].

After Three Gorges Dam is constructed, the Three Gorges Reservoir (TGR) forms a new vast hydro-fluctuation belt with an elevation of 145 m in summer to 175 m in winter, a length of more than 2000 km and an area of 300 km² [20, 21], which has provided unique ecological habitats for those diverse species in the TGR area [22]. Many field surveys have shown that most of the pre-dam riparian vegetation is gradually dying out due to the inability to adapt to the reversal of submergence time, the prolongation of flood duration and the new hydrological fluctuation zone (up to 30m in elevation) [23]. Generally, plants use limited oxygen and light under flood conditions, resulting in production of excessive reactive oxygen species (ROS) [24], which were the key factors that hindered the growth and development of submerged plants [25, 26]. They are forced to undergo the oxidative pathway [27], and usually develop an antioxidant defense system consisting of some antioxidant enzymes and specific metabolites to convert these excessive ROS into harmless products in order to protect themselves [28, 29].

As symbionts, endophytic fungi can produce antioxidants, block the chain reaction of ROS to help host plants respond to various biotic and abiotic stresses [9, 30]. Some studies have also showed that endophytes can increase the survival rate of host plants during flooding stress by producing antioxidants independently [31, 32]. Severe oxidative damage of free radicals has been confirmed to be associated with various diseases, including cancer, inflammation, aging and neurodegenerative diseases [33]. It has been advised that antioxidants should be warranted in the enhancement of human health [34, 35]. Currently, the demand for natural antioxidants from endophytic fungi has been increasing along with the finding that natural antioxidants have fewer side effects on human health than artificially synthesized substances [36, 37]. Additionally, the search for safer and novel drugs based on the natural product from endophytes is of utmost importance because of the increasing incidence of cancer and the recently emerged, rapid evolution of superbugs due to antibiotic resistance [38, 39].

After Three Gorges Dam is constructed, many abiotic stresses in the natural habitat strongly influence plant growth and development, such as summer flooding, infertility, drought, salinity and soil erosion etc. So far, only a few highly tolerant plants have been reported to survive, which include Salix variegata, Morus alba L., Myricaria laxiflora [22, 40]. Among them, Distylium chinense (Fr.) Diels, a rare evergreen ornamental shrub of Hamamelidaceae family known for the beautiful flowers (Fig. 1a), is a native species to the riparian wetland in the TGR area of the Yangtze River and its tributaries [20, 41, 42]. Since 2005, D. chinense was considered as an ideal choice for solid embankment after the construction of the Three Gorges Dam owing to its strong root system, erosion tolerance, strong flooding tolerance and resistance to sand burial soaks [43]. Several biological studies have been made for D. chinense such as morphological characteristics, natural habitat, genetic diversity, community structure, ecological adaptability, reproductive allocation and propagation methods [42, 44, 45]. It should be noted that the roots of D. chinense has been used in traditional Chinese medicine and folk medicine as an analgesic, anti-inflammatory and diuretic [46]. However, there is no information on the diversity and bioactive potential of endophytes community from D. chinense. Thus, the aim of this study was to provide the first evidence of endophytic fungi diversity within the D. chinense, provide a working collection of endophytes and investigate endophytes with antioxidant, antimicrobial and anticancer activities in order to explore the potential sources of novel drugs.

Methods

Plant material

Three healthy and asymptomatic D. chinense plants were randomly collected from different locations on an island in the Banan district (N29°42′45.63″, E106°60′69.43″) of Chongqing of China in the Three Gorges Reservoir area in October 2014. All plant materials were immediately sent to the laboratory and stored in a refrigerator at 4°C.
Each sample tissues were used within 24 h after collection. The plant samples were identified as *D. chinense* by Prof. Hongping Deng and were preserved in Chongqing Key Laboratory of Plant Resource Conservation and Germplasm Innovation, School of Life Science, Southwest University, Chongqing 400715, China.

**Isolation and cultivation of endophytic fungi**
The surface sterilization and isolation of fungal endophytes were carried out, and some improvements were made [47]. In the first instance, all stems and roots of plant materials were thoroughly washed in running tap water to remove debris and then air-dried naturally in the clean bench. Clean tissue pieces were disinfected in series of solutions: 75% ethanol; sterile distilled water; 0.1% mercuric chloride (HgCl) (v/v). Finally, they were again rinsed with sterile distilled water three times. After surface sterilization, the tissues were dried on blotting sheets, cut into 0.5 cm lengths and transferred to potato dextrose agar (PDA) medium supplemented with 60 mg/mL of streptomycin and 100 mg/mL of ampicillin using an aseptic technique to inhibit the bacterial growth. At the same time, the final sterile water used for washing the tissues (100 μL) was also plated on the PDA to confirm the sterilization effect of the surface. The inoculated plates were incubated at 28°C in darkness for 2-15 days to allow the growth of endophytic fungal hyphae and checked regularly. Pure isolates were checked for purity and transferred to another PDA plate by the hyphal tip method [48]. The obtained endophytic fungal isolates were coded according to their source tissues (DR1-1, DR1-2, DR2-1, etc. from roots and DS2-1, DS3-1, DS1-2, etc. from the stems). These endophytes were classified according to colony color, form, elevation and margin characteristics on PDA. Based on the groupings, strains with different morphology were screened for molecular identification.

**Molecular identification and phylogenetic evaluation of endophytic fungi**
According to the above simple classification, each type of fungi was chosen as the representative for molecular biological identification using the fungal genomic deoxyribonucleic acid (DNA) extraction. Fungal genomic DNA extraction was previously described by Landum et al. according to the manufacturer’s instructions using the DNeasy Plant Minikit (Qiagen, Germany) [49]. The nuclear ribosomal DNA internal transcribed spacer
(ITS) of the fungal isolates were amplified by forward primer, ITS1-F (5′-TCCGTAGGTAACCGTGCGG-3′) and reverse primer, ITS4 (5′-TCTCCGAGGTATTGATATGC-3′) [50]. The final reaction volume was 25 μL, containing 12.5 μL of 2X PCRBIO Taq Mix Red (PCR Biosystems, UK), 0.4 μM of forward and reverse primers and 10 ng of genomic DNA template. For negative control, the DNA was replaced with distilled water to verify absence of contamination. PCR was carried out using MyCycler™ (Bio-Rad, USA), programmed for 5 min 94°C; 30 cycles for 30s at 94°C, 60s at 55°C, and 1min at 72°C; and a final 10 min extension at 72 °C. The PCR products were separated using 1% agarose gel in 1X TAE buffer (90mM Tris-acetate and 2 nM EDTA, pH 8.0), with ethidium bromide (0.5μg/mL) staining and recorded with FluorChemTM (Alpha Innotech, USA). The PCR products were sequenced by Invitrogen Co. Shanghai.

In phylogenetic evaluation, the ITS DNA sequences and downloaded sequences of their nearest neighbors were aligned in Alignment Explorer of MEGA 4 software using ClustalW option [51, 52]. MUSCLE (UPGMA) algorithm was used to prune and verify the sequence. The evolutionary distances and history were calculated by means of Neighbor-Joining methods [53]. The robustness of the trees were assessed by bootstrap analysis with 1000 replication [54].

Bioactivity evaluation

Fermentation and preparation of fungal extract

Fermentation and preparation of the fungi were determined according to the scheme proposed by Ya-Li et al. with some modifications [55]. Briefly, all isolates were cultured in potato dextrose broth (PDB, the medium contained potato 200 g and glucose 20 g in 1 L of purified water) for 14 d at 28 °C on a shaker at 180 r/min. Crude fermentation broth was filtered with eight layers of gauze. Filtered liquid was extracted three times with the same amount of ethyl acetate. The organic solvent extract was then evaporated under reduced pressure to yield an ethyl acetate extract. The ethyl acetate extracts were dissolved in methanol and the final concentration was 10 mg/mL for bioactivity screening.

Antioxidant activity

The radical scavenging ability was evaluated by using adapted 2,2′-diphenyl-1-picrylhydrazyl (DPPH) method described previously with some modification [56]. Thus, an aliquot of extract (50 μL) was added to 150 μL of methanol DPPH (50 μM). The reaction mixture was transferred to a 96-well microtiter plate and incubated at room temperature for 30 min in the dark and absorbance was measured at 517 nm using a microtiter plate reader (Bio-Rad 680, BIO-RAD, USA). Ascorbic acid (Vc) and methanol were used as positive and negative controls, respectively. Meanwhile, three experimental replicates were taken for the assay.

Antimicrobial activity

The determination of antimicrobial activity was based on the disk diffusion method with some modification [57]. Each disc (Oxford cup, 6 mm diameter) contained 200 μg of endophytic fungi extraction (10 mg/mL). The indicator organisms included gram-negative: Escherichia coli (ATCC25922, EC), Pseudomonas aeruginosa (CMCC(B)10104, PA); gram-positive: Staphylococcus aureus (ATCC6538, SA), Bacillus subtilis (ATCC6633, BS); three pathogenic fungi Penicillium (ATCC9080, P), Aspergillus niger (CMCC(F)98003, AN) and Candida albicans (CMCC(F)98001, CA). There were purchased from Shanghai Luwei Technology Co., Ltd. Streptomycin and amphotericin B were used as positive controls and methanol as negative control. The antimicrobial activities were determined according to diameters of inhibitory zones (ZI) and experiments were repeated three times.

Anticancer activity

Human papillary thyroid carcinoma cell line IHH4 and human pancreatic adenocarcinoma cell line CFPA-C-1 were obtained from the Cell Line Bank of the Chinese Academy of Science. The anticancer activity was determined according to CCK-8 assay [58]. Cisplatin was used as the positive control and repeated for three times.

Isolation of bioactive metabolites

Based on the results of the above antioxidant, antimicrobial and anticancer activities, the strain Irpex lacteus DR10-1 was selected for the chemical analysis because it exhibited widest broad-spectrum bioactivities. Irpex lacteus DR10-1 culture filtrate 14L was fermented by the same method as above mentioned. Crude ethyl acetate (EtOAc) extracts from Irpex lacteus DR10-1 (67g) was obtained and further purified by a silica gel column (200-300 mesh, 4.0 × 70 cm, with 70 g of silica gel), and eluted with gradient mixtures of petroleum ether (60-90 °C) and EtOAc to yield 5 fractions (A1-A5). Fraction A2 (156 mg) was further purified by a silica gel column chromatography (300-400 mesh, 2.0 × 25 cm, with 15 g of silica gel) and eluted with gradient mixtures of chloroform (CHCl3) and EtOAc to yield compound 1 (30mg). Fraction A4 (98 mg) was further purified by a silica gel column chromatography (300-400 mesh, 1.0 × 25 cm, with 35 g of silica gel), and eluted with gradient mixtures of CHCl3 and methanol (MeOH) to obtain compound 2 (25mg).

Nuclear magnetic resonance (NMR) spectra were recorded by Bruker Ascend 500 spectrometer. The spectrometer operated at 500 MHz for 1H nuclei and 125 MHz for 13C nuclei. Chemical shift was quoted in parts...
per million (ppm), referring to the appropriate residual solvent peak.

**Statistical Analysis**

Using species as the statistical unit, the number of isolates (N) and the isolation frequency (IF) for each endophytic fungal species in different tissues or the total plant (Additional file 1 Table S1) were calculated. Species richness index (S) and Margalef index (D’ ) were used to evaluate species richness, which were two important parameters for alpha diversity analysis [59]. Shannon-Wiener index (H’) and Simpson’s diversity index (Ds) were used to the species diversity, respectively [60, 61]. Additionally, the Jaccard Similarity Index (JC) was used to compare the species composition of the stem and root tissues [62]. Results were expressed as mean ± standard deviation (SD) of triplicate of measurements for the DPPH and CCK-8 assays. Data were conducted with SPSS 18.0 for Windows (SPSS Inc., Chicago, USA).

**Results**

**Community composition and abundance**

A total of 154 fungal endophytes were isolated from *D. chinense* plants collected from the TGR area. Among them, 30 different representative morphospecies were determined according to cultural characteristics (Fig. 1b). Of these detected, 30 isolates were categorized into 27 different taxa (Ascomycota, 19; Basidiomycota, 8), and further into nine distinct orders (Fig. 1c). The Fig. 2 showed the phylogenetic tree of 30 fungal strains isolated from the NCBI database and the accession numbers of the matched rDNA-ITS sequences. The supplementary table data (Additional file 1: Table S1) provided detailed information on 30 representative strains, including their sources and isolation frequencies.

At the order level, the Diaporthales possessed the most taxa, six taxa, accounting for 22.2% of the total fungal taxa and they had 48 isolates, around 31.2% of the total fungal isolates (Fig. 1d). Conversely, the Botryosphaeriales had the most isolates, 52 isolates, accounting for 33.8% of the total fungal isolates and they possessed four species, around 14.8% of the total fungal species. The Polyporales and Agaricales were the second and third most abundant orders with high species, and together constituted approximately 33.3% of all the species. Analogously, the Xylariales and Polyporales were the second and third most abundant isolates, and together constituted approximately 18.1% of all the isolates. The other identified orders were the Hypocreales, Microascales, Eurotiales and Discellaceae, which together constituted approximately 22.2% and 10.4% of all species and isolates, respectively (Fig. 1d). Interestingly, the most common fungal species between roots and stems were *Phomopsis* sp. (24.7%), followed by *Neofusicoccum parvum* (23.4%). However, *Phomopsis* were not from order with highest isolate rates.

**Species diversity and richness abundance of fungi**

The richness and species diversity of culturable endophytic fungi were significantly higher in stems than in roots (Table 1). Among the 27 total taxa, 16 (59.3% of total) were obtained from the stems. A total of 3 fungal taxa- *Neofusicoccum parvum*, *Phomopsis* sp. and *Discellaceae* sp. were distributed in both plant tissues, but ten taxa-*Fusarium sp.*, *Fusarium equiseti*, *Xyilaria venosa*, *Lasiodiplodia theobromae*, *Penicillium ochrochloron*, *Rhizoctonia bataticola*, *Robillarda sessilis*, *Coprinellus xanthothrix*, *Polyporus crassa* and *Irpex lacteus* were only found in the roots (Fig. 3). Similarly, of the nine orders, two were found in both stems and roots, but the Hypocreales, Xylariales, Eurotiales and Discellaceae were unique to the roots (Fig. 4). Additionally, Shannon-Wiener index (H) and Simpson diversity index (Ds) could be used to analyze species diversity. Generally, the higher the Shannon’s diversity index (usually between 1.5 and 4.5), the closer the Simpson’s diversity index is to 1, the stronger the adaptability of the community to the change of micro environment is, and the community presents the trend of expanding the distribution range and entering the new environment [63]. On the other hand, the species richness (S) and Margalef index (D’) can reflect the richness of endophytic fungi species. The larger the values of S and D’ were, the richer the species of endophytic fungi were [64]. As shown in Table 1, the species richness and diversity of endophytic fungi in stems were higher than those in roots, and the values of S (16), D’ (3.5802), H’ (2.5323) and Ds (0.8659) were higher. In addition, the similarity index (Jaccard’s index) was used to estimate the similarity between stem and root. Although stem and root samples collected in TRG field were adjacent to each other and lived in the same place, the Jaccard’s index only showed 0.11 between stems and roots, showing low similarity. These indices showed that endophytic fungi in different tissues had significant diversity.

**Bioactivity evaluation of fungal endophytes**

As mentioned above, one of the main purposes of this study was to identify endophytic fungi that could be cultured and applied to develop their potentially beneficial properties for plants and humans. All 154 fungal endophytes isolated from *D. chinense* at TGR were evaluated for their antioxidant, antimicrobial and anticancer activities (Additional file 1: Table S2-S4). Among the 154 isolates, 99 (64.3%), 153 (99.4%) and 27 (17.5%) fungal extracts showed antioxidant activity, antimicrobial activity against at least one indicator organisms and
Fig. 2 Phylogeny analyses of endophytic fungi from *D. chinense*. The tree was derived by neighbor-joining methods analysis of ITS1–5.8S–ITS4 sequences [53] and 30 sequences retrieved from Gen Bank. The percentage of replicate trees in which associated taxa were clustered together in the bootstrap test (1000 replicates, values below 50% are not shown) are shown next to the branches. Phylogeny analyses were conducted in MEGA 4 software [51, 52].
Table 1 Diversity analyses of endophytic fungi from D. chinense

| Diversity Index          | Different Tissues | Total |
|--------------------------|-------------------|-------|
|                          | Root   | Stem  |
| Species richness (S)     | 14     | 16    | 27   |
| Margalef index (D’)      | 2.9109 | 3.5802| 5.1619|
| Shannon-Wiener index (H’)| 2.1828 | 2.5323| 2.4824|
| Simpson diversity index (Ds)| 0.8366 | 0.8659| 0.8646|
| Jaccard’s indice (JC)     | 0.11   |       |

Characterization of metabolites of strain DR10-1

Among 154 strains recovered from D. chinense, the EtOAc extract of the culture broth of Irpex lacteus DR10-1 (Additional file 2: Figure S1-S2) exhibited higher antioxidant activity than that of ascorbic acid, antimicrobial capability by inhibiting the growth of seven tested pathogens and showed anticancer activity against both tested cancer cell lines, and was subjected to column chromatography over silica gel, Sephadex LH-20 to afford two known compounds. The structures of the two known compounds were established as indole-3-carboxylic acid (compound 1) [65] (Additional file 2: Figure S3-S4) and indole-3-carboxaldehyde (compound 2) [66] (Additional file 2: Figure S5-S6) by comparing their spectroscopic data with those in the literature (Fig. 6).

Discussion

Considering the roles of endophytic fungi in plant development, growth, adaptability and diversity, we needed to fill this gap in order to exploit of endophytes for a better understanding of D. chinense plant and their important metabolites found in the TGR. Therefore, one of the purposes of this study was to examine the community composition of fungal endophytes from TGR. Here, we...
took a culture-dependent approach, since our final goal was to build a working collection of fungal endophytes that could be explored for their potentially beneficial properties in *D. chinense* plant. In this work, a total of 154 endophytic fungi were isolated from *D. chinense* in the TGR and classified into 27 different taxa according to their morphological characteristics and unique phenotypic characters. The identified fungi were mainly composed of *Phomopsis*, *N. parvum*, *Diaporthe*, *Fusarium* and *Irpex* with relative frequencies 24.7%, 23.4%, 3.2%, 5.2% and 4.5%, respectively. Among them, fungi that belong to *Phomopsis*, *Diaporthe*, *Fusarium* and *Irpex* have...
been reported as the main endophytes of wetland shrub Myricaria laxiflora in the TGR [67] and riparian plant species [68]. Additionally, Fusarium, Phomopsis and Irpex has also been reported to be not sensitive to flooding stress [67]. By contrast, other several genera, including Penicillium ochrochloron, Mycorrhizal basidiomycete, Ceriporia lacerta, Diaporthe longicolla, Diaporthe eres, Flavodon flavus, Irpex sp., Parphoma sp. and Phoma medicaginis, were only isolated from D. chienense with low relative abundance. Even so, the existence of these minor genera has been demonstrated to play an important ecological role in their host plants as reported [69].

According to the literatures, fungal endophytic community of land plant mainly belonged to Sordariomycetes, Dothideomycetes and Pezizomycetes fungi while plants from water or moist environments were more often parasitized by Eurotiomycetes [70–72]. In the current study, the most prevalent class was Sordariomycetes with relative frequency of 50%, followed by Dothideomycetes and Eurotiomycetes at 33.8% and 1.3%, respectively. Obviously, both terrestrial and aquatic fungi are present in the D. chinense plant. This was in accordance with the report by Kandalepas et al., who discovered high numbers of Sordariomycetes and low numbers of Dothideomycetes and Eurotiomycetes in wetland plants from Louisiana [71].

Among these isolates found in D. chinense, many from the genera Phomopsis, Fusarium, Diaporthe, Neofusicoccum parvum, Xylaria venosula, Lasiodiplodia theobromae and Botryosphaeria dothidea have been reported as common pathogenic fungi in some wild and cultured plants [75]. For example, Diaporthe and Phomopsis complex were the causes of seed decay and cause soybean blight and canker diseases [78]; Neofusicoccum parvum was reported as one of the most aggressive causal agents of the trunk disease Botryosphaeria dieback [79]; Botryosphaeria and its anamorph complex were particularly important for symptoms such as fruit rot, shoot blight, dieback and canker of numerous woody hosts [80]. Although the symptoms of disease did not appear in D. chinense plant collected, as reported, these fungi might switch their lifestyles from a mutualistic to parasitic interaction which depended on genetic factors of both partners [81], imbalance in nutrient exchange [82] and environmental variations [83, 84]. Furthermore, the interaction type between an endophyte and a host plant also could be modulated if the plant was subjected to physiological stress [85]. It has been shown that individual fungal species which could switch lifestyles might represent an evolutionary transition, or simply fungi that had achieved remarkable ecological plasticity, might ensure the optimal growth and reproduction in a variety of hosts, which ultimately would lead to the expansion of...
hindered remarkable antioxidant activity, of which 18
and they showed at least one biological activity. Among the screened isolates, 99 (64.3%) isolates ex-
hibited remarkable antioxidant activity, of which 18
(11.7%) had very notable activity with IC50 value of ≤ 3 μg/mL, suggesting that it may protect D. chinense
from oxidative stress in the flooding environment as suggested by Zeng et al [88]. Because of the protect-
effective of antioxidants, they are essential for plant
survival and fitness and presumably selection have
lead to both redundant and highly specific path-
ways that address ROS production and stress medi-
ation [89]. For example, Mirzhosseinei et al. have
reported that endophytic fungi can alleviate the oxida-
tive damage produced by ROS accumulation in plant
cells such as F. arundinacea [90, 91]. Regarding anti-
microbial activity, 31.2%, 11.7%, 19.5%, 69.5% and
29.9% extracts of endophytes showed activity against
Penicillium, Candida albicans, Aspergillus niger,
Staphylococcus aureus and Escherichia coli respect-
ively, which was comparable and even exceeded some
results reported by other authors in similar studies
[92, 93]. For example, from the 39 endophytic fungal
extracts of Viguiera arenaria and Tithonia Diversifolia
plants, Guimaraes et al. found only 5.1% and 25.6%
extracts to be active against Staphylococcus aureus
and Escherichia coli respectively [94]. Unexpectedly,
Pseudomonas aeruginosa was most sensitive to the
fungal extracts among the tested bacterial though it
was reported to be drug resistant towards many anti-
biotics [95]. Usually, the fungal extracts also showed
higher activity against the Gram-negative than the
Gram-positive ones. This different sensitivity has been
suggested to be attributed to the high level of lipo-
polysaccharides that are contained in the Gram-
positive bacteria membrane, which could make the
cell wall impermeable to bioactive compounds [96].
As for anticancer activity, 27 out of 154 fungal exactly
(17.5%) showed activity against IHH4/CFPAC-1 cell
line, in which 11 fungal extracts were active against
both tested cell lines. Statistically, 18 out of 27 anti-
cancer isolates were exclusively isolated from the
roots, 9 were only recovered from stems. Generally,
for the same fungal species e.g. Neofusicoccum par-
vum, the isolates from roots showed stronger bio-
activity compared to those from the stems regardless
of antimicrobial, antioxidant or anticancer bioactiv-
ities. Such data well supported the traditional practice
of native people who often used the extracts from
roots to relieve analgesic, antirheumatic and diuretic
[43].

Of these isolates screened, a high proportion of bioac-
tivities were mostly detected from the fungal extracts be-
longing to Phomopsis sp. (24.7%), Neofusicoccum parvum (23.4%) and Xylaria venosula (9.1%), which was
attributed to their high separation rate. As did here, Pho-
mosis sp. have been reported as dominant member of
the endophytic community [97]. Phomopsis is a domin-
ant member of the endophytic community because it
grows rapidly, thus inhibiting slow growing endophytes,
which might be one of the reasons for the low number
of species detected in this study [98]. Additionally, Pho-
mosis and related taxa contain important endophytic
and are known to produce a series of bioactive second-
ary metabolites in vitro with a variety of different chem-
ical structures [99]. However, few studies conducted on
the active metabolites of Neofusicoccum parvum, and its
antioxidant activity accounted for the highest proportion
in the current study, which has never been reported in
previous studies [100, 101]. Besides, Xylaria species are
widely distributed on the temperate to the tropical zones
in the terrestrial globe, and fungi of this genus have been
proved to be potential sources of novel secondary me-
tabolites, and many of them have biological activities re-
lated to drug discovery, including cytotoxic, antimalarial,
and antimicrobial activities [102]. In terms of bioactivity,
active extracts of DS16-1 (Phomopsis sp.), DR28-1 (Pho-
mopsis sp.), DS35-1 (Cerioporia lacerata), DR41-2 (Ceri-
poria lacerata) and R46-1 (Phomopsis sp.) were found
promising. In particular, the strain DR10-1(Irpex lacteus)
showed wide spectrum bioactivities, suggesting that pos-
sible use of one endophyte could be a valuable candidate
as new antioxidant, antimicrobial and anticancer agents.

Finally, we isolated two known compounds including
indole-3-carboxylic acid and indole-3-carboxylic acid der-
ivatives from the wide spectrum bioactive strain L. lac-
teus DR10-1. As far as we know, this was the first time
that indole-3-carboxylic acid (1) and indole-3-
carboxaldehyde (2) had been isolated from endophytic
fungus Irpex lacteus. It was previously demonstrated
that indole-3-carboxylic acid isolated from endophytic
fungal strain of Epicoccum nigrum associated with
Entada abyssinica had remarkable activity against Gram-
negative strains (Staphylococcus aureus) with MIC
values of 6.25 μg/mL [103]. This finding was consistent
with literature report on the antibacterial activity of
indole-3-carboxylic acid, from which a novel series of
indole-3-carboxylic acid derivatives were previously reported to possess potent antibacterial activity against *Enterococcus faecalis* [104]. In addition, it has been reported that indole-3-carboxylic acid had weak cytotoxic effects on both normal and tumor cells, and its antioxidant activity is weak [103]. Recently, the indole-3-carboxylic acid (IAA) and other auxins have been shown to stimulate cell elongation, resulting in root growth initiation or an enhancement of nutritional elements absorption by the hosts [105, 106]. Besides, IAA was supposed to improve the adaptability of plant microbe interaction [107].

**Conclusions**

The study provided insight into the diversity of endophytic fungal community isolated from *D. chinense* growing in the TGR area. This was the first report where studies on the diversity of endophytic fungi that inhabited *D. chinense* plant growing in the TGR area had been carried out. The data obtained showed that of the 154 endophytic fungal extracts screened for antioxidant, antimicrobial and anticancer potential. Among the 154 isolates tested, most of the endophytic fungal extracts showed abundant bioactivity. Specifically, the *I. lacteus* DR10-1 strain exhibited significant antioxidant, antimicrobial and anticancer potential. By expanding fermentation *I. lacteus* DR10-1 strain, two active secondary metabolites, indole-3-carboxylic acid (1) and indole-3-carboxaldehyde (2), were obtained, and they showed abundant biological activities. Therefore, we had for the first time reported its extract for bioactivity such as antioxidant, antimicrobial and anticancer potential. It was demonstrated that it could harbor metabolites that could serve as promising antioxidant, antimicrobial and anticancer agents.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s12866-019-1634-0.

**Additional file 2: Figure S1.** Morphological characteristics and microscopic morphology of DR10-1. **Figure S2.** Neighboring-clone tree based on ITS rDNA sequence of the fungus DR10-1 and its closest ITS rDNA matches in the GenBank. **Figure S3.** 1H NMR spectrum of compound 1 in CD3COCD3. **Figure S4.** 13C and DEPT NMR spectrum of compound 2 in CD3COCD3. **Figure S5.** 1H NMR spectrum of compound 2 in CD3COCD3.

**Abbreviations**

AN: Aspergillus niger; BS: Bacillus subtilis; CA: Candida albicans; CHCl3: Chloroform; D': Margalef Index; DNA: Deoxyribonucleic Acid; DPPH: 2,2′-diphenyl-1-picrylhydrazyl; Ds: Simpson’s Diversity Index; EC: Escherichia coli; EtOAc: Ethyl Acetate; H′: Shannon-Wiener Index; HyGCl: Mercuric Chloride; IF: Isolation Frequency; ITS: Internal Transcribed Spacer; JC: Jaccard Similarity Index; MeOH: Methanol; N: Number of isolates; NMR: Nuclear Magnetic Resonance; P: Penicillium; PA: Pseudomonas aeruginosa; PDA: Potato Dextrose Agar; PDB: Potato Dextrose Broth; ROS: Reactive Oxygen Species; S: Species Richness Index; SA: Staphylococcus aureus; SD: Standard Deviation; TGR: Three Gorges Reservoir; Vc: Ascorbic Acid; ZI: Inhibitory Zones

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**Authors’ contributions**

XXD contributed to the experimental conception and design, the process of the whole project for carrying out the related experiments, data analysis, and manuscript draft; FFX contributed to the fungal isolate culture and data analysis; DQ, TCG, WYS, SHZ, BHY, JRX, YJP, contributed reagents, materials, and analysis tools, JYD contributed to the experimental design as well as the manuscript draft and improvement. All authors read and approved the final manuscript.

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**Availability of data and materials**

Sequences obtained in this study were deposited in the NCBI GenBank database (For accession numbers refer Table S1 of the Additional file 1). Other datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Ethics approval and consent to participate**

No specific permission was required for the described study area. The research work doesn’t involve any endangered or protected plant species.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Song HC, Qin D, Han MJ, Wang L, Zhang K, Dong JY. Bioactive 2-pyrones metabolites from an endophytic *Phomopsis asparagi* SWUKS2020 of *Kashiua angustifolia*. Phytochem Lett. 2017;22:235–40.
50. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pcr Protocols. 1994; 38:315–22.

51. Tamura K, Dudley J, Nei M, Kumar S. MEGAS: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007; 24: 1596-1599.

52. Edgar R. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792–7.

53. Koichi T, MasatoshI N, Sudhir K. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA. 2004;101:11030–5.

54. Kaur J, Bhambri P, Gupta OP. Distance based Phylogenetic Trees with Bootstrap. International Journal of Computer Applications. 2013;47:6–10.

55. Li YI, Zhang FS, Chen J, Cui IL, Xing YM, Li XQ, Guo SX. Diversity and antimicrobial activity of endophytic fungi associated with the alpine plant Saussurea involucrata. Biol Pharm Bull. 2010;33:1300.

56. Zhao J, Fu Y, Luo M, Zu Y, Wang W, Zhao C, Gu C. Endophytic fungi from pigeon pea (Cajanus cajan) (L.) Millip.) produce antioxidiant Cajanistilbene J. Agric Food Chem. 2012;60:4314–9.

57. Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. J Ethnopharmacol. 1989;23:127–49.

58. Sun W, Lan X, Wang Z, Dong W, He L, Zhang T, Zhang P, Zhang H. MicroRNA-144 inhibits proliferation by targeting WW domain-containing transcription regulator protein 1 in papillary thyroid cancer. Oncology Lett. 2018; 15: 1007-1013.

59. Zhang G, Zhang J, Yang L, Zhang L, Jiang D, Chen W, Li G. Diversity and biocatalytic potential of endophytic fungi in Bussicia napa. Biol Control. 2014;72:98–108.

60. Santos JM, Correia VG, Phillips AJL. Primers for mating-type diagnosis in Neotyphodium. Curr Genet. 1998;32:680–4.

61. Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW, Crous PW. Translation of conidial wall proteins in filamentous ascomycetes. Mycol Res. 2004;108:1561–5.

62. Venkatachalam A, Thirunavukkarasum S, Suryanarayanan TS. Distribution and diversity of endophytes in seagrasses. Fungal Ecol. 2015;17:30–6.

63. Li PQ, Wu Z, Liu T, Wang YN. Biodiversity, Phylogeny, and Antifungal Functions of Endophytic Fungi Associated with Zanthoxylum bungeanum. Int J Mol Sci. 2016; 17(10):1541.

64. Jiang S, Wang W, Xue X, Cao C, Zhang Y. Fungal diversity in major oil-shale mines in China. Environ Geochem Health. 2017;39:253–60.

65. Xiao HT, Liu B, Hao XY, Yang XS, Sun QY. Chemical constituents from Micromonospora gloriae. J Nat Prod. 2009;72:697–703.

66. Maria-Teresa GL, Woldemichael G, Singh MP, Suarez PA, Maiese WM. Isolation of three new naturally occurring flavonoids from Saussurea deltoidea. Chem Nat Compd. 2009;45:539–40.

67. Tian W, Bi YH, Zeng W, Jiang W, Xue YH, Wang GX, Liu SP. Diversity of endophytic fungi from Viguiera arenaria and Tithonia diversifolia extracts of endophytic fungi isolated from Viguiera arenaria and Tithonia diversifolia. Mycobiology. 2012;40:8–12.

68. An HM, Liu Y, Zhao XF, Huang Q, Yuan SH, Zhang D, Dong JY. Characterization of cadmium-resistant endophytic fungi from Salix xanthophila Franch. in Three Gorges Reservoir Region, China. Microbiol Res. 2015;167:929–37.

69. Weishampel PA, Bedford BL. Wetland dicots and monocots differ in colonization by arbuscular mycorrhizal fungi and dark septate endophytes. Mycorrhiza. 2006; 16:495–502.

70. Stevens KJ, Wellner MR, Acevedo MF. Dark septate endophyte and arbuscular mycorrhizal status of vegetation colonizing a bottomland hardwood forest after a 100 year flood. Aquat Bot. 2010;92:105–11.

71. U'Ren JM, Lutzoni F, Miadlikowska J, Laetsch AD, Arnold AE. Host and Geographic Structure. Microb Ecol. 2014;67:735–46.

72. Hamilton CE, Helder M, Saikkonen K. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Divers. 2012;54:1–7.

73. Gebreyohannes G, Moges F, Gebreyes H. Endophytic fungal communities in woody perennials of three tropical deciduous forests in Ethiopia. Eur J For Pathol. 1993;23:51–63.

74. Akello J, Dubois T, Gold CS, Coyne D, Nakavuma J, Paparu P. Beauveria bassiana (Balsamo) vulliemoen as an endophyte in tissue culture banana (Musa spp.). J Invertebr Pathol. 2007; 98:34–42.

75. Hendry SJ, Boddy L, Lansdále D. Abiotic variables effect differential expression of latent infections in bees (Fagus sylvatica). New Phytol. 2002; 155:449–60.

76. Kogel RH, Franken P, Hückelhoven R. Endophyte or parasite-what decides? Eur J Plant Pathol. 2005;112:123–31.

77. Rodriguez R, Redman R. More than 400 million years of evolution and some plants still can’t make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot. 2008;59:1109–14.

78. Kogel RH, Franken P, Hückelhoven R. Endophyte or parasite-what decides? Curr Opin Plant Biol. 2006;9:358–63.

79. Richter CE, Schenone AL, Lomas D. Abiotic variables effect differential expression of latent infections in bees (Fagus sylvatica). Curr Opin Plant Biol. 2006;9:358–63.

80. Halmschlager E. Endophytic fungi in leaves and twigs of Quercus petrea. J Exp Bot. 1999;50:23–31.

81. Akello J, Dubois T, Gold CS, Coyne D, Nakavuma J, Paparu P. Beauveria bassiana (Balsamo) vulliemoen as an endophyte in tissue culture banana (Musa spp.). J Invertebr Pathol. 2007; 98:34–42.

82. Kogel RH, Franken P, Hückelhoven R. Endophyte or parasite-what decides? Curr Opin Plant Biol. 2006;9:358–63.

83. Hendry SJ, Boddy L, Lansdále D. Abiotic variables effect differential expression of latent infections in bees (Fagus sylvatica). New Phytol. 2002; 155:449–60.

84. Halmschlager E. Endophytic fungi in leaves and twigs of Quercus petrea. J Exp Bot. 1999;50:23–31.

85. Hendry SJ, Boddy L, Lansdále D. Abiotic variables effect differential expression of latent infections in bees (Fagus sylvatica). New Phytol. 2002; 155:449–60.

86. Halmschlager E. Endophytic fungi in leaves and twigs of Quercus petrea. J Exp Bot. 1999;50:23–31.

87. Rukachaisirikul V, Arunpanichlert J, Sukpondma Y, Phongpaichit S, Sakayaroy J. Metabolites from the endophytic fungi Botryosphaeria rhodina PSU-M35 and PSU-M114. Tetrahedron. 2009;65:10590–5.

88. Micromonospora gloriae. J Nat Prod. 2009;72:697–703.

89. Phongpaichit S, Rungjindamai N, Sakayaroy J. ABC transporter inhibitors isolated from the endophytic fungus Trichoderma hamatum isolate DIS 21B8 promotes growth and delays the onset of the drought response in Theobroma cacao. J Exp Bot. 2009;60:3279–95.

90. Mirzahossini Z, Shabani L, Sabzalian MR, Sharifi-Tehrani M. Antimicrobial activity of endophytic fungi associated with the alpine plant Macrophytes: Diverse Host-Generalists Characterized by Tissue Preferences and Geographic Structure. Microb Ecol. 2014;67:735–46.

91. Mirzahossini Z, Shabani L, Sabzalian MR, Sharifi-Tehrani M. ABC transporter inhibitors isolated from the endophytic fungus Trichoderma hamatum isolate DIS 21B8 promotes growth and delays the onset of the drought response in Theobroma cacao. J Exp Bot. 2009;60:3279–95.

92. Singh DK, Sharma VK, Kumar J, Mishra A, Verma SK, Sieber TN, Kharwa RN. Diversity of endophytic mycobionta of tropical tree Tectona grandis Linn.f. Spatiotemporal and tissue type effects. Sci Rep. 2017; 7: 3745.
99. Abreu LM, Costa SS, Pfenning LH, Takahashi JA, Larsen TO, Andersen B. Chemical and molecular characterization of Phomopsis and Cytospora-like endophytes from different host plants in Brazil. Fungal Biol-UK. 2012;116:249–60.

100. Massonnet M, Morales-Cruz A, Figueroa-Balderas R, Lawrence DP, Baumgartner K, Cantu D. Condition-dependent co-regulation of genomic clusters of virulence factors in the grapevine trunk pathogen Neofusicoccum parvum. Mol Plant Pathol. 2018;19:21–34.

101. Alberti I, Prodi A, Nipoti P, Grassi G. First report of Neofusicoccum parvum causing stem and branch canker on Cannabis sativa in Italy. J Plant Dis Protect. 2018;125:511–3.

102. Song F, Wu SH, Zhai YZ, Xuan QC, Wang T. Secondary metabolites from the genus Xylaria and their bioactivities. Chem Biodivers. 2014;11:673–94.

103. Dzoyem JP, Melong R, Tsamo AT, Maffo T, Kapche DGWF, Ngadjui BT, McGaw LJ, Bloff JN. Cytotoxicity, antioxidant and antibacterial activity of four compounds produced by an endophytic fungus Epicoccum nigrum associated with Entada abyssinica. Rev Bras Farmacogn. 2017;27:251–3.

104. Himaja M, Jose T, Remana MV, Anand R, Munisajasekhar D. SYNTHESIS AND BIOLOGICAL EVALUATION OF INDOLE-3-CARBOXYLIC ACID DERIVATIVES OF AMINO ACIDS AND PEPTIDES. International Research Journal of Pharmacy. 2010;1:436–40.

105. Meena KK, Sorty AM, Bilia UM, Choudhary K, Gupta P, Pareek A, Singh DP, Prabh P, Sahu PK, Gupta VK, Singh HB, Krishanani KK, Minhas PS. Abiotic Stress Responses and Microbe-Mediated Mitigation in Plants: The Omics Strategies. Front Plant Sci. 2017;8:172.

106. Hartley SE, Gange AC. Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol. 2009;54:323–42.

107. Chowdhury MEK, Jeon J, Rim SO, Park YH, Lee SK, Bae H. Composition, diversity and bioactivity of culturable bacterial endophytes in mountain-cultivated ginseng in Korea. Sci Rep. 2017;7:10098.

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