Sains Malaysiana 51(6)(2022): 1687-1696
http://doi.org/10.17576/jsm-2022-5106-07

Diversity and Antibacterial and Antioxidant Activities of Fungal Endophytes from the Roots of *Eucalyptus deglupta*

(Kepelbagaian serta Aktiviti Antibakteria dan Antioksidan Endofit Kulat daripada Akar *Eucalyptus deglupta*)

WEI WANG[†,1], ZILING MAO[†,1], CHUNYIN WU[1], HAMZA SHAHID[1], SHENGKUN WANG[2,*] & TIJIANG SHAN[1]

[1]*College of Forestry and Landscape Architecture, South China Agricultural University, Guangdong Guangzhou, 510642, China*
[2]*Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou 510520, China*

Received: 1 January 2021/Accepted: 22 November 2021

[†]*These authors contributed equally to this work.*

ABSTRACT

In this study, 45 endophytic fungal strains were isolated from the roots of *Eucalyptus deglupta*. Among them, 16 distinct strains were identified and classified into 14 different genera (*Celoporthe, Aspergillus, Castanediella, Chaetomium, Biscogniauxia, Sordariales, Pestalotiopsis, Clitopilus, Cylindrocladiella, Calonectria, Trichoderma, Xylaria, Neofusicoccum* and *Pleosporales)* according to their morphological characteristics and molecular information. The genera *Aspergillus* and *Calonectria* were the dominant endophytic fungi in the roots of *E. deglupta.* In addition, the antibacterial and antioxidant activities of the 16 endophytic fungi isolated from the roots of *E. deglupta* were evaluated. All the strains displayed inhibitory activities against *Agrobacterium tumefaciens, Bacillus subtilis, Escherichia coli,* and *Xanthomonas vesicatoria*. Strains Edf-1 to Edf-4, Edf-11 and Edf-12 demonstrated strong inhibitory activity against *R. solanacearum* with plaque diameters between 5 and 10 mm. The crude extract of Edf-14 had inhibitory activity against all tested bacteria. Five strains, Edf-1 to Edf-5, demonstrated a strong scavenging capacity for 2,2-diphenyl-1-picrylhydrazyl (DPPH), with IC_{50} values of 0.26 ± 0.04, 0.11 ± 0.03, 0.20 ± 0.05, 0.10 ± 0.04 and 0.14 ± 0.02 mg/mL, respectively. Hence, endophytic fungi isolated from the roots of *E. deglupta* showed antibacterial and antioxidant activities, providing a theoretical foundation for further isolation and identification of specific active components.

Keywords: Antibacterial activity; antioxidant activity; crude extracts; endophytic fungi; *Eucalyptus deglupta*

ABSTRAK

Dalam kajian ini, 45 strain kulat endofit telah dipencilkan daripada akar *Eucalyptus deglupta*. Sejumlah 16 strain yang ternyata berbeza di antaranya telah dikenal pasti dan dikelaskan kepada 14 genera berbeza (*Celoporthe, Aspergillus, Castanediella, Chaetomium, Biscogniauxia, Sordariales, Pestalotiopsis, Clitopilus, Cylindrocladiella, Calonectria, Trichoderma, Xylaria, Neofusicoccum* dan *Pleosporales*) berdasarkan ciri morfologi dan maklumat molekul. Genera *Aspergillus* dan *Calonectria* adalah kulat endofit yang dominan dalam akar *E. deglupta.* Di samping itu, aktiviti antibakteria dan antioksidan bagi kesemua 16 kulat endofit yang dipencilkan daripada akar *E. deglupta* telah dinilai. Kesemua strain menunjukkan aktiviti perencatan terhadap *Agrobacterium tumefaciens, Bacillus subtilis, Escherichia coli* dan *Xanthomonas vesicatoria*. Strain Edf-1 hingga Edf-4, Edf-11 dan Edf-12 telah menunjukkan aktiviti perencatan yang kuat terhadap *R. solanacearum* dengan menghasilkan plak berdiameter antara 5 dan 10 mm. Ekstrak kasar Edf-14 mempunyai aktiviti perencatan terhadap semua bakteria yang diuji. Sebanyak lima strain iaitu Edf-1 hingga Edf-5 telah menunjukkan kapasiti penghapusan radikal yang kuat oleh 2,2-difenil-1-pikrylhidrazil (DPPH), dengan IC_{50} masing-masing bernilai 0.26 ± 0.04, 0.11 ± 0.03, 0.20 ± 0.05, 0.104, 0.104 dan 0.11. 0.14 ± 0.02 mg/mL. Oleh itu, kulat endofit yang dipencilkan daripada akar *E. deglupta* telah menunjukkan aktiviti antibakteria dan antioksidan yang menyediakan asas teori bagi kajian lanjutan untuk memencilkan dan mengenal pasti komponen aktif yang khusus.

Kata kunci: Aktiviti antibakteria; aktiviti antioksidan; ekstrak mentah; *Eucalyptus deglupta;* kulat endofit

Introduction

Plant endophytic fungi are microorganisms present in living plant tissues that do not harm host plants but rather play a vital role in their protection against pathogens, animals and harsh environmental conditions. They are considered a source of abundant secondary metabolites with significant biological activities (Mollaei et al. 2019). Endophytic fungi produce identical secondary metabolites as the host plants with similar biological activities (Zhao et al. 2017). To some extent, studies on the biological activity of endophytic fungi could reduce the large-scale utilisation of plants to protect the natural environment. Endophytic fungi have many prospects because of their biodiversity and bioactive metabolites (Tiwari et al. 2018; Wu et al. 2019). Various research has shown that endophytic fungi are a vital resource of bioactive secondary metabolites which possess antibacterial, anticancer, antiviral, insecticidal and antioxidant activities and phytoremediation potential (Chi et al. 2019; Ferdous et al. 2019; Palanichamy et al. 2018; Pansanit & Pripdeevech 2018; Ye et al. 2019). Diverse endophytic fungi have been reported to produce bioactive compounds such as alkaloids (Mohamadi et al. 2018; Patel et al. 2012), terpenoids (Yuan et al. 2018), phenols (Jacobo-Velázquez & Cisneros-Zevallos 2020; Sahni et al. 2020), peptides (Jiang et al. 2013; Song et al. 2019), steroids (Kim & Shim 2019; Miao et al. 2019), lactones (Kaaniche et al. 2019) and pyrenes (Kim & Shim 2019).

Bacterial wilt caused by *Ralstonia solanacearum* is one of the most serious diseases of *Eucalyptus* spp. in China (Wang et al. 2014). However, efficient control measures for this disease remain limited. *Eucalyptus* spp. are important fast-growing timber and economical tree species that possess antibacterial, cytotoxic, antifungal, and high allelopathic activities (El-Rokiek et al. 2019; Miguel et al. 2017; Syukri et al. 2020; Tiwari et al. 2018). *Eucalyptus* spp. are rich in flavonoids and phenolics, also a potential source of novel antibacterial and antioxidant compounds (Nasr et al. 2019; Nwabor et al. 2019; Shang et al. 2019). In the previous study, four new depsidones together with nine known compounds were isolated from the endophytic fungus *Chaetomium* sp. Eef-10 associated with *E. exserta,* and demonstrated different antibacterial, cytotoxic, anticancer, and antioxidant activities (Ouyang et al. 2020, 2018). Ten endophytic fungal strains were isolated from *E. citriodora* where Ecf-4 (*Rhytidhysteron* sp., MK211261) and Ecf-1 (*Epicoccum* sp., MK211258) displayed the most vigorous activity against *R. solanacearum* (Shan et al. 2019).

However, the secondary metabolites of endophytic fungi in *E. deglupta* have rarely been studied. In this study, the endophytic fungi from the roots of *E. deglupta* were isolated and identified, and the antibacterial and antioxidant activities of secondary metabolites produced by the endophytic fungi were also evaluated. This study aimed to screen endophytic fungal strains with significant biological activity and provides theoretical guidance for the prevention and control of bacterial wilt of *Eucalyptus* spp. as well as prospects for further separation and identification of specific active components.

Materials and Methods

Sample preparation

Healthy roots of *E. deglupta* were collected from the Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou, China, in October 2015. The taxonomic identification of the plant materials was performed by Dr. Shengkun Wang of the Research Institute of Tropical Forestry, Chinese Academy of Forestry. One Gram-positive (*Bacillus subtilis* ATCC11562) and five Gram-negative (*Agrobacterium tumefaciens* ATCC11158, *Escherichia coli* ATCC25922, *Pseudomonas lachrymans* ATCC11921, *R. solanacearum* ATCC11696 and *Xanthomonas vesicatoria* ATCC11633) strains were provided by the College of Forestry and Landscape Architecture of South China Agricultural University for the antibacterial assay.

Isolation and purification of the endophytic fungi

The endophytic fungi were isolated using the method from our previous report (Shan et al. 2019). After separation and purification, the purified strains were stored at 4 °C on the inclined surface of potato dextrose agar (PDA) in a 5 mL cryopreservation tube. The colonisation frequency (CF) of each isolate was determined as CF = $(N_{COL}/N_t) \times 100$, where N_{COL} is the number of each fungus and N_t is the total number of segments, according to the method of Hata and Futai (1995).

Morphological identification

The morphological characteristics and identification were conducted according to a standard taxonomic key including colony diameter, texture, color and the dimensions and morphology of hyphae and conidia (Ainsworth et al. 1973). First, the edge hyphae of the endophytic fungi were inoculated onto the PDA medium, and cultures were grown in darkness at 28 °C for 3-20 days. Then, the cultures were identified based on the colony morphology.

MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS

Genomic DNA of the endophytic fungal strains was extracted using a DNA extraction kit. A total of 3 µL of DNA extract, 1 µL of ITS4 and ITS5, and 25 µL of DNA were mixed, and 20 µL of sterile deionised water were added to the PCR tube. Universal primers for endophytic fungi, ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′), were used for ITS-rDNA amplification as reported previously (Shan et al. 2019). The PCR amplification procedure started with pre-denaturation at 94 ℃ for 3 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 ℃ for 30 s, and extension at 72 °C for 8 min. The amplification was completed by holding the reaction mixture at 72 °C for 10 min to allow complete extension of PCR products. The PCR products were stored at 4 °C. The sequences were sequenced and analysed using the BLAST program against the National Center for Biotechnology Information database and submitted to GenBank, where the accession numbers were obtained. After MAFTT (version 7) processing, MEGA7 software was used to construct a phylogenetic tree with the maximum likelihood method.

PREPARATION OF ENDOPHYTIC FUNGAL CRUDE EXTRACTS

Different crude extracts of endophytic fungi were prepared according to the method of Ouyang et al. (2020). Briefly, the endophytic fungi were cultivated on PDA in Petri dishes for 3 to 5 days. The mycelia were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of potato glucose liquid medium (5 flasks for each fungal culture). Seed cultures were injected into sterilized rice medium (25 g per flask) and stored for 60 days at 25 °C. Later, the fermentation product was soaked in EtOAc for 7 days. This process was repeated three times. Finally, the fermentation liquid was filtered using vacuum with reduced pressure until concentrated to dryness. The crude extract was collected into a clean penicillin bottle for future use and stored at 4 to 5 °C.

ANTIBACTERIAL ACTIVITY ASSAY

Thin-layer chromatography (TLC)-bioautography assays for different endophytic fungal crude extracts were carried out as described previously (Shan et al. 2018). All the crude extracts were dissolved with a mixture of reagents, and the appropriate solution was extracted using a 0.5 mm capillary tube and sampled on a TLC plate. The developing agent used for TLC was petroleum ether:acetone (4:1, V/V). Then, 0.2 mg/mL streptomycin sulfate at the origin point on one side of the TLC plate was used as the positive control. A certain amount of prepared bacterial solution (150 mL LB + 10 mL bacterial solution) was added to sterilised LB semisolid medium (agar concentration of 0.5%), and the mixture was oscillated and homogenised. The prepared bacterial suspension was evenly inoculated onto a silica gel plate with a pipette. Bacterial inoculated TLC plates were placed at 28 ℃ for 12 h. After 12 h, color developing agent MTT was sprayed uniformly on the TLC plates. The experimental results were observed after approximately 2 min. The bacterial growth inhibition area was white and the other areas were blue and purple. Antibacterial activity was detected as white inhibition zones against a purple background and the R_f value of the antibacterial area was determined. $R_f = D_1/D_2$ where D_1 is the distance between the antimicrobial area and the initial sample point, and D_2 is the distance between the developing solvent front and initial sample point on a TLC plate. At the same time, the diameter of each antibacterial area was measured.

ANTIOXIDANT ACTIVITY ASSAY

All the crude extracts were tested for the antioxidant activity based on the reduction of a methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described previously (Ouyang et al. 2018). First of all, 20 mg of DPPH was accurately weighed and dissolved in 100 mL of absolute ethanol. Dimethyl sulfoxide (DMSO) was used to configure the samples of different crude extracts with an initial concentration of 20 mg/mL, and then the solutions with concentrations of 10 mg/mL to 0.15625 mg/mL were successively diluted by the half dilution method. The initial concentration of the positive control BHT was 2 mg/mL, and later, the solution was diluted from 1 to 0.15625 mg/mL using the same method. Second, 80 µL of DPPH and 20 µL of sample solution were added to a 96 microwell plate, and each concentration gradient was replicated three times. Sealing film was used to create an airtight cover, the light absorption value was measured at 517 nm by horizontal oscillation for 10 min, and then the mixtures were heated in a water bath at 37 ℃ for 30 min. A total of 20 µL DMSO solution was used as a negative control instead of sample solution, and the treatment was repeated three times. The inhibition (%) of free radicals (DPPH) was calculated as $[(A_{control} / A_{sample})/ A_{control}] \times 100$, where $A_{control}$ is the absorbance of the negative control, and A_{sample} is the absorbance of each crude extracts. Then, the results from all replicates were plotted and analysed using Excel. The logarithm (X) of the sample concentration was taken, and the clearance rate was converted to a probability (Y). Finally, the regression equation ($Y = aX + B$) and IC_{50} value of antioxidant activity were obtained.

Results and Discussion

Forty-five endophytic fungal strains were isolated from the roots of *E. deglupta*. According to morphological characteristics and molecular identification results, the 16 strains shown in Figure 1 and Table 1 were identified successfully and assigned to 14 genera namely *Aspergillus*, *Celoporthe*, *Castanediella*, *Chaetomium*, *Biscogniauxia*, *Sordariales*, *Pestalotiopsis*, *Clitopilus*, *Cylindrocladiella*, *Calonectria*, *Trichoderma*, *Xylaria*, *Neofusicoccum,* and *Pleosporales*. After submitting their ITS4-5.8S-ITS5 partial sequences to the GenBank, accession numbers (KX960786 - KX960801) and the closest related species were obtained. All the sequenced strains had more than 99% similarity with the closest related species in GenBank (Table 1). The primary fungal isolates were *Aspergillus* sp. and *Calonectria* sp., with CFs of 20.0 and 13.3%, respectively, as shown in Table 1.

All the genera were grouped into clades in the phylogenetic tree based on ITS sequences. They were categorised into 8 groups which are Xylariomycetidae, Diaporthomycetidae, Hypocreomycetidae, Sordariomycetidae, Eurotiomycetidae, Botryosphaeriales, Pleosporomycetidae and Agaricomycetidae (Figure 2). It has been proven that endophytic fungi are related to the growing site, climate, tissues, organs and age of the host plant (Miguel et al. 2017). The present study was conducted to isolate endophytic fungi from the roots of *E. deglupta* for the first time. It may also be important to study the endophytic fungi of *E. deglupta* under different environmental conditions. Sixteen endophytic fungi were successfully identified and divided into 14 different genera based on ITS sequencing, reflecting the diversity of endophytic fungi from the roots of *E. deglupta*.

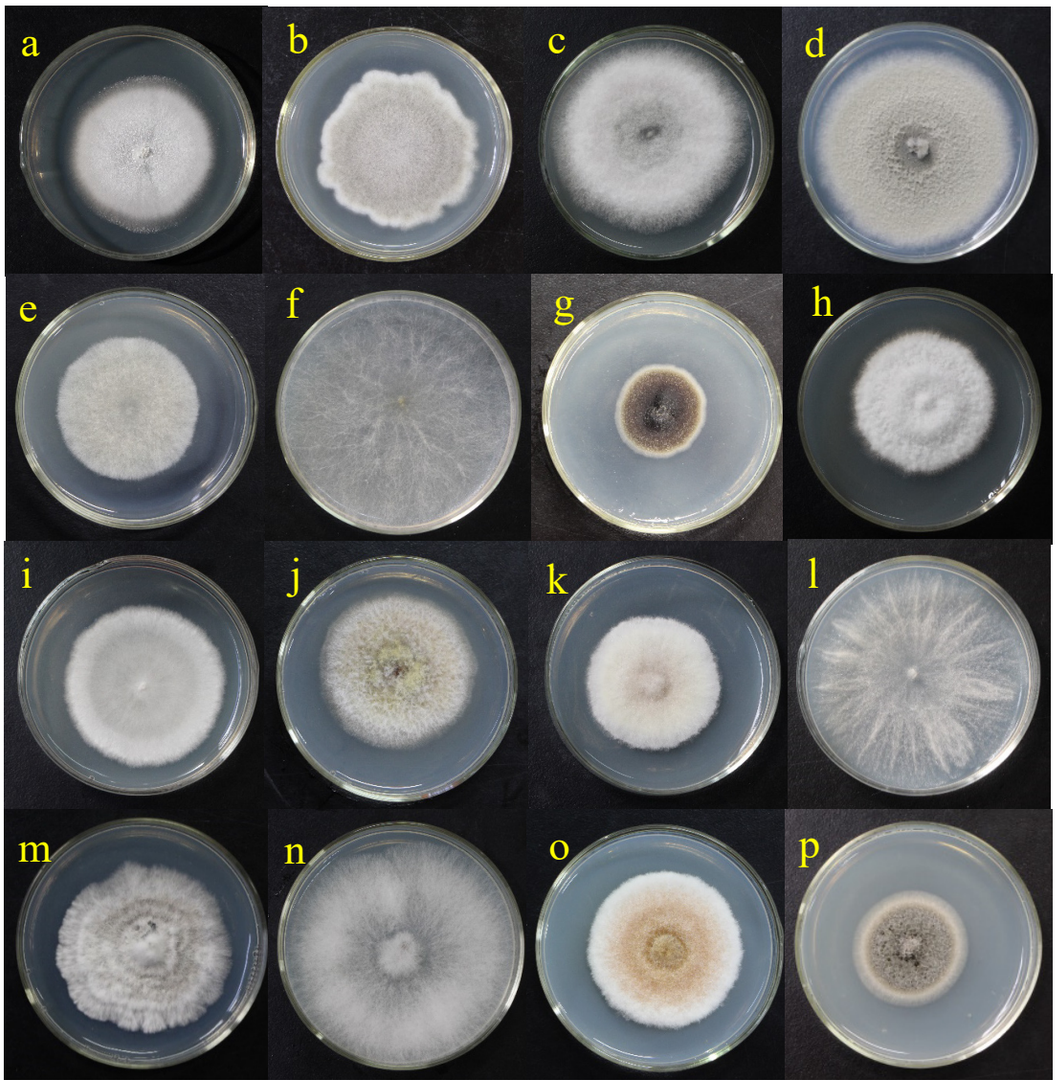

FIGURE 1. Endophytic fungal colonies isolated from the roots of *Eucalyptus deglupta*

Note: a□p was Edf-1□Edf-16, respectively

TABLE 1. Endophytic fungi and their closest relatives based on the data from BLAST analysis

Fungal isolate	CF (%)	GenBank accession number	Micro and microscopic identification	Closest related species	Similarity (100%)
Edf-1	11.1	KX960786	*Aspergillus* sp.	MK734050.1 *Aspergillus hiratsukae*	100
Edf-2	8.9	KX960787	*Aspergillus* sp.	MK793770.1 *Aspergillus* sp.	100
Edf-3	6.7	KX960788	*Celoporthe* sp.	MN172406.1 *Celoporthe* sp.	100
Edf-4	8.9	KX960789	*Castanediella* sp.	MH860269.1 *Castanediella couratarii*	100
Edf-5	8.9	KX960790	*Chaetomium* sp.	MH858130.1 *Chaetomium globosum*	100
Edf-6	4.4	KX960791	*Biscogniauxia* sp.	EU009960.1 *Biscogniauxia* sp.	99
Edf-7	6.7	KX960792	*Sordariales* sp.	JX243892.1 *Sordariales* sp.	100
Edf-8	2.2	KX960793	*Pestalotiopsis* sp.	HQ608091.1 *Pestalotiopsis* sp.	100
Edf-9	2.2	KX960794	*Clitopilus* sp.	EU273512.1 *Clitopilus prunulus*	98
Edf-10	2.2	KX960795	*Cylindrocladiella* sp.	MN701712.1 *Cylindrocladiella* sp.	99
Edf-11	8.9	KX960796	*Calonectria* sp.	GQ280561.1 *Calonectria chinensis*	99
Edf-12	4.4	KX960797	*Trichoderma* sp.	KT336515.1 *Trichoderma harzianum*	100
Edf-13	2.2	KX960798	*Xylaria* sp.	KP133322.1 *Xylaria apiculata*	99
Edf-14	2.2	KX960799	*Neofusicoccum* sp.	KJ657701.1 *Neofusicoccum parvum*	100
Edf-15	4.4	KX960800	*Calonectria* sp.	NR137039.1 *Calonectria cerciana*	100
Edf-16	2.2	KX960801	*Pleosporales* sp.	KU593608.1 *Pleosporales* sp.	99

In this study, a TLC-bioautography assay was conducted to evaluate the antibacterial activity of secondary metabolites produced by endophytic fungi. This assay was a rapid screening method for secondary metabolites with antibacterial activity mainly according to the diameters of inhibition spots and the R_f values (Shan et al. 2018). The inhibitory activity values of 16 different crude extracts are listed in Table 2. All the crude extracts showed antibacterial activity against *X. vesicatoria*, and the plaque diameters of Edf-7, Edf-9, Edf-11 to Edf-14 and Edf-16 were between 5 and 10 mm. Strain Edf-14 displayed inhibitory activity against all the tested bacteria. Edf-14 belonging to *Neofusicoccum* sp. exhibited a broad spectrum of antibacterial activity and had excellent inhibitory activity against bacterial strains. Strains Edf-1 to Edf-4, Edf-11 and Edf-12 demonstrated strong inhibitory activity against *R. solanacearum* with plaque diameters between 5 and 10 mm. Strain

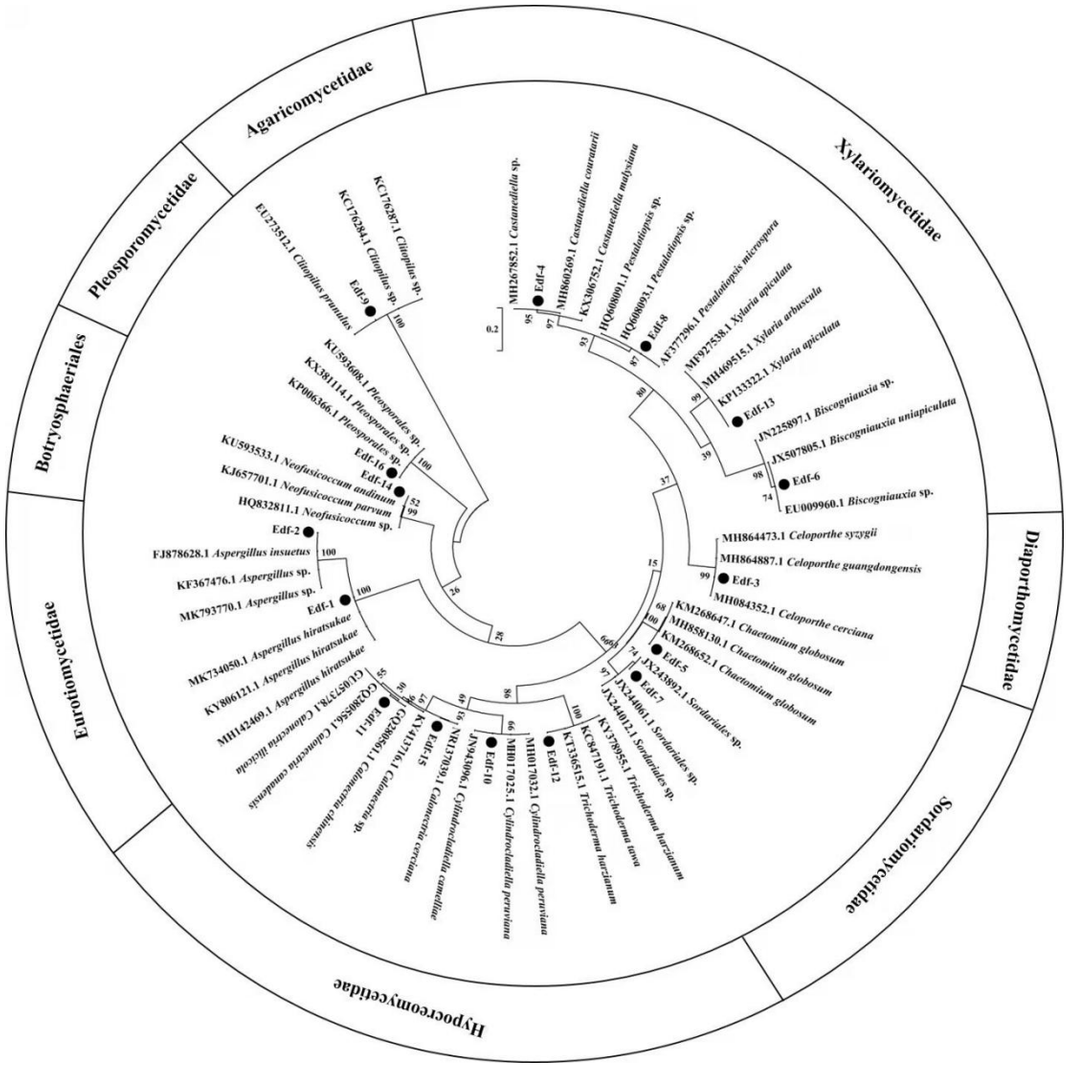

FIGURE 2. Phylogenetic tree of endophytic fungi from the roots of *Eucalyptus deglupta* based on rDNA-ITS sequence

Edf-11 (*Castanediella* sp.) had strong antibacterial activity against *R. solanacearum*, *A. tumefaciens*, and *X. vesicatoria*. To date, there have been no relevant studies on the secondary metabolites of *Castanediella* sp. Edf-2 displayed the strongest inhibitory activity against *A. tumefaciens,* and the plaque diameter was greater than 10 mm. Most of the strains lacked inhibitory activity against *P. lachrymans*, with the exceptions of Edf-11 and Edf-14. Edf-8 showed the weakest antibacterial activity and had only weak inhibitory activity against *X. vesicatoria*. The R_f value is closely related to the polarity of the compound. In this study, the antibacterial activity in the R_f range of 0.20 to 0.50 was stronger than that in the R_f range of 0.00 to 0.20. This antibacterial assay result indicated that the secondary metabolites produced by the endophytic fungi of *E. deglupta* were mainly intermediate-polarity substances. In addition, inhibitory activity of different endophytic fungal crude extracts did not differ between Gram-positive and Gram-negative bacteria.

TABLE 2. Antibacterial activity of different crude extracts extracted from endophytic fungi Edf-1~Edf-15

Fungal isolate	*A. tumefaciens*	*R. solanacearum*	*X. vesicatoria*	*B. subtilis*	*P. lachrymans*	*E. coli*
Edf-1	0.00-0.15^{++}	0.00-0.17^{++}	0.00-0.10^{+}	0.00-0.17^{+}; 0.38-0.42^{+}	-	-
Edf-2	0.00-0.22^{++}; 0.25-0.28^{+++}; 0.38-0.43^{+}	0.00-0.20^{++}; 0.23-0.27^{++}	0.00-0.17^{+}; 0.23-0.25^{+}	0.00-0.17^{+}; 0.22-0.25^{++}	-	0.00-0.07^{+}; 0.38-0.47^{+}
Edf-3	0.00-0.07^{+}; 0.15-0.20^{+}; 0.30-0.53^{++}	0.25-0.42^{++}	0.00-0.15^{+}; 0.25-0.38^{+}	0.00-0.38^{+}	-	0.00-0.32^{++}; 0.42-0.58^{++}
Edf-4	0.00-0.20^{++}; 0.42-0.48^{+}	0.00-0.23^{++}	0.00-0.18^{+}	0.00-0.27^{+}; 0.45-0.48^{++}	-	-
Edf-5	0.00-0.18^{+}; 0.47-0.52^{+}	0.00-0.18^{+}	0.00-0.12^{+}	0.12-0.17^{+}; 0.42-0.53^{++}	-	-
Edf-6	-	0.00-0.08^{+}	0.00-0.12^{+}	0.07-0.18^{++}; 0.08-0.10^{+}	-	-
Edf-7	0.00-0.18^{+}; 0.25-0.37^{++}	0.33-0.43^{+}	0.00-0.22^{+}; 0.27-0.35^{++}	0.33-0.40^{+}	-	0.33-0.37^{+}
Edf-8	-	-	0.00-0.07^{+}; 0.17-0.25^{+}	-	-	-
Edf-9	0.00-0.18^{++}	0.00-0.18^{+}	0.00-0.08^{++}; 0.17-0.27^{+}	0.00-0.18^{+}	-	0.00-0.18^{++}; 0.48-0.52^{+}
Edf-10	0.00-0.20^{++}; 0.42-0.53^{+}	0.00-0.10^{+}	0.00-0.05^{+}	-	-	-
Edf-11	0.00-0.52^{++}	0.00-0.37^{++}	0.00-0.18^{++}; 0.23-0.42^{++}	0.00-0.13^{+}; 0.38-0.43^{+}	0.00-0.03^{+}; 0.37-0.45^{+}	-
Edf-12	0.00-0.28^{++}; 0.38-0.43^{+}	0.00-0.23^{++}	0.00-0.33^{++}	0.00-0.17^{++}; 0.25-0.32^{+}	-	0.00-0.17^{++}; 0.23-0.32^{+}
Edf-13	0.00-0.15^{+}	-	0.00-0.12^{++}; 0.15-0.18^{+}	0.00-0.12^{+}	-	0.00-0.08^{+}; 0.17-0.23^{+}
Edf-14	0.00-0.07^{+}	0.00-0.10^{+}	0.00-0.17^{++}	0.00-0.12^{++}	0.00-0.10^{++}	0.00-0.08^{+}
Edf-15	0.00-0.15^{+}	0.28-0.32^{+}	0.00-0.17^{+}	0.00-0.05^{+}	-	-
Edf-16	0.00-0.18^{+}	0.40-0.43^{+}	0.00-0.08^{++}	-	-	0.00-0.05^{+}
Streptomycin sulphate	++	++	+++	+++	+++	+++

Note: -, antimicrobial activity was not observed; +, the diameter of the antimicrobial activity area was 0-5 mm; ++, the diameter of the antimicrobial activity area was 5-10 mm; +++, the diameter of the antimicrobial activity area was more than 10 mm; the positive control was streptomycin sulfate which was only sampled on the TLC plate and showed antibacterial activity

The antioxidant activity of 15 different crude extracts from all the isolates except Edf-16 was determined by DPPH free radical scavenging assay (Figure 3). Crude extracts of Edf-1 (*Aspergillus* sp.), Edf-2 (*Aspergillus* sp.), Edf-3 (*Celoporthe* sp.), Edf-4 (*Castanediella* sp.) and Edf-5 (*Chaetomium* sp.) showed excellent antioxidant activities with the IC_{50} values of 0.26 ± 0.04, 0.11 ± 0.03, 0.20 ± 0.05, 0.10 ± 0.04 and 0.14 ± 0.02 mg/mL, respectively. In previous reports, it was found that the secondary metabolites from *Chaetomium* sp. had strong antioxidant activities (Tantapakul et al. 2020; Wang et al. 2019; Yao et al. 2019). Edf-10, Edf-13 and Edf-14 displayed weak antioxidant activity with high IC_{50} values (> 2.00 mg/mL). In addition, the rest of the extracts showed IC_{50} values from 0.50 to 2.00 mg/mL, such as that from Edf-6, with a value of 0.50 mg/mL.

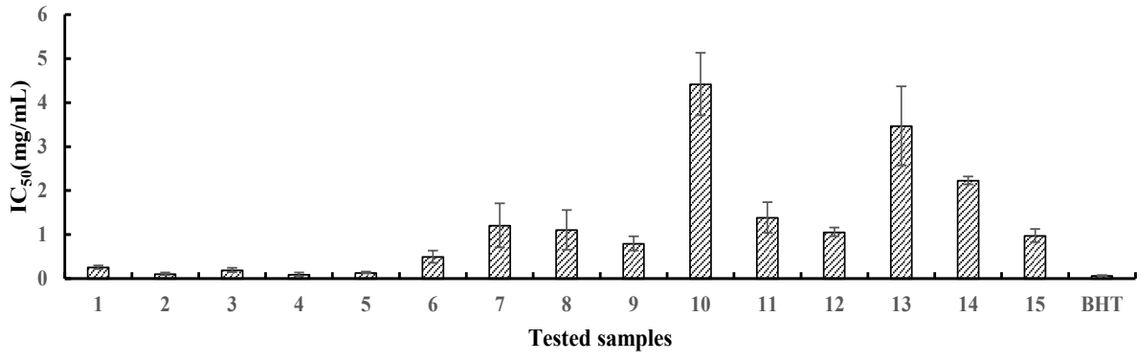

FIGURE 3. Antioxidant activities of 15 crude extracts. Samples 1 to 15 were the crude extracts of Edf-1~Edf-15, respectively

Conclusions

This study presented the identification and biological activities of endophytic fungi from the economic tree *E. deglupta* for the first time. Endophytic fungi from the roots of *E. deglupta* distributed in 14 different genera where *Aspergillus* sp. and *Calonectria* sp. were the dominant strains. Endophytic fungi Edf-1 to Edf-5, Edf-11 and Edf-12 showed obvious bioactivity. These strains have the potential to be pharmaceuticals that inhibit bacterial wilt of *Eucalyptus* spp. or other pathogenic bacteria as well as exhibited antioxidant activity. They could be used as candidate strains for further isolation of active compounds. This study provided an important theoretical basis for the large-scale development and application of biological pesticides and biological control of diseases.

Acknowledgements

We thank Huixiong Wu for logistical assistance. This research was co-financed by the Natural Science Foundation of Guangdong Province (2019A1515011554), the National Natural Science Foundation of China (32071766), the Forestry Science and Technology Innovation Project of Guangdong Province (2020KJCX004) and the Key Research and Development Projects of Guangdong Province (2020B020214001).

References

Ainsworth, G.C., Sparrow, F.K. & Sussman, A.S. 1973. A taxonomic review with keys ascomycetes and fungi imperfecti. In *The Fungi, An Advanced Treatise*. 4th ed. New York: Academic Press. pp. 100-121.

Chi, W.C., Pang, K.L., Chen, W.L., Wang, G.J. & Lee, T.H. 2019. Antimicrobial and iNOS inhibitory activities of the endophytic fungi isolated from the mangrove plant *Acanthus ilicifolius* var. *xiamenensis*. *Botanical Studies* 60(1): 1-8.

El-Rokiek, K.G., Dawood, M.G., Sadak, M.S. & El-Awadi, M.E.I.S. 2019. The effect of the natural extracts of garlic or *Eucalyptus* on the growth, yield and some chemical constituents in quinoa plants. *Bulletin of the National Research Centre* 43(1): 1-7.

Ferdous, K.J., Afroz, F., Islam, M.R., Mazid, M.A. & Sohrab, M.H. 2019. Isolated endophytic fungi from the plant *Curcuma longa* and their potential bioactivity - A review. *Pharmacology & Pharmacy* 10(5): 244-270.

Hata, K. & Futai, K. 1995. Endophytic fungi associated healthy pine needles infested by the pine needle gall midge, *Thecodiplosis japonensis*. *Canadian Journal of Botany* 73(3): 384-390.

Jacobo-Velázquez, D.A. & Cisneros-Zevallos, L. 2020. Bioactive phenolics and polyphenols: Current advances and future trends. *International Journal of Molecular Sciences* 21(17): 6142.

Jiang, W., Ye, P.P., Arthur Chen, C.T., Wang, K.W., Liu, P.Y., He, S., Wu, X.D., Gan, L.S., Ye, Y. & Wu, B. 2013. Two novel hepatocellular carcinoma cycle inhibitory cyclodepsipeptides from a hydrothermal vent crab-associated fungus *Aspergillus clavatus* C2WU. *Marine Drugs* 11(12): 4761-4772.

Kaaniche, F., Hamed, A., Abdel-Razek, A.S., Wibberg, D., Abdissa, N., EI Euch, I.Z., Allouche, N., Mellouli, L., Shaaban, M. & Sewald, N. 2019. Bioactive secondary metabolites from new endophytic fungus *Curvularia*. sp isolated from *Rauwolfia macrophylla. PLoS ONE* 14(6): e0217627.

Kim, J.W. & Shim, S.H. 2019. The fungus *Colletotrichum* as a source for bioactive secondary metabolites. *Archives of Pharmacal Research* 42(9): 735-753.

Miao, F.P., Zuo, J.C., Liu, X.G. & Ji, N.Y. 2019. Algicidal activities of secondary metabolites of marine macroalgal-derived endophytic fungi. *Journal of Oceanology and Limnology* 37(1): 112-121.

Miguel, P.S.B., Delvaux, J.C., de Oliveira, M.N.V., Moreira, B.C., Borges, A.C., Tótol, M.R., Neves, J.C.L. & Costa, M.D. 2017. Diversity and distribution of the endophytic fungal community in eucalyptus leaves. *African Journal of Microbiology Research* 11(3): 92-105.

Mollaei, S., Khanehbarndaz, O., Gerami-Khashal, Z. & Ebadi, M. 2019. Molecular identification and phytochemical screening of endophytic fungi isolated from *Lithospermum officinale* L. roots: A new source of shikonin. *Phytochemistry* 168(3): 112116.

Mohamadi, N., Sharififar, F., Pournamdari, M. & Ansari, M. 2018. A review on biosynthesis, analytical techniques, and pharmacological activities of trigonelline as a plant alkaloid. *Journal of Dietary Supplements* 15(2): 207-222.

Nasr, A., Khan, T.S. & Zhu, G.P. 2019. Phenolic compounds and antioxidants from *Eucalyptus camaldulensis* as affected by some extraction conditions, a preparative optimization for GC-MS analysis. *Preparative Biochemistry and Biotechnology* 49(5): 1-13.

Nwabor, O.F., Vongkamjan, K. & Voravuthikunchai, S.P. 2019. Antioxidant properties and antibacterial effects of *Eucalyptus camaldulensis* ethanolic leaf extract on biofilm formation, motility, hemolysin production, and cell membrane of the foodborne pathogen *Listeria monocytogenes. Foodborne Pathogens and Disease* 16(8): 581-589.

Ouyang, J.K., Wu, C.Y., Wang, Y.Y., Zhang, C.B., Mao, Z.L. & Shan, T.J. 2020. Identification of endophytic fungus *Chaetomium* sp. Eef-10 from *Eucalyptus exserta* and analysis of its active ingredients. *Journal of South China Agricultural University* 41(2): 104-110.

Ouyang, J.K., Mao, Z.L., Guo, H., Xie, Y.Y., Cui, Z.H., Sun, J., Wu, H.X., Wen, X.J., Wang, J. & Shan, T.J. 2018. Mollicellins O-R, four new depsidones isolated from the endophytic fungus *Chaetomium* sp. Eef-10. *Molecules* 23(12): 3218.

Palanichamy, P., Krishnamoorthy, G., Kannan, S. & Marudhamuthu, M. 2018. Bioactive potential of secondary metabolites derived from medicinal plant endophytes. *Egyptian Journal of Basic and Applied Sciences* 5(4): 303-312.

Pansanit, A. & Pripdeevech, P. 2018. Antibacterial secondary metabolites from an endophytic fungus, *Arthrinium* sp. MFLUCC16-1053 isolated from *Zingiber cassumunar*. *Mycology* 9(4): 264-272.

Patel, K., Gadewar, M., Tripathi, R., Prasad, S.K. & Patel, D.K. 2012. A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid ''Harmine''. *Asian Pacific Journal of Tropical Biomedicine* 2(8): 660-664.

Sahni, T., Sharma, S., Arora, G. & Verma, D. 2020. Synthesis, characterization and antifungal activity of a substituted coumarin and its derivatives. *Pesticide Research Journal* 32(1): 39-48.

Shan, T.J., Feng, H., Xie, Y.Y., Shao, C., Wang, J. & Mao, Z.L. 2019. Endophytic fungi isolated from *Eucalyptus citriodora* Hook. f. and the antibacterial activity of their crude extracts. *Plant Protection* 45(6): 149-155.

Shan, T.J., Zhang, W., Wang, S., Bai, R., Weng, D., Lin, X. & Sun, J. 2018. Detection of the active ingredients from 26 plants using TLC-bioautography assay. *Plant Protection* 44(6): 66-72.

Shang, Z.C., Han, C., Xu, J.L., Liu, R.H., Yin, Y., Wang, X.B., Yang, M.H. & Kong, L.Y. 2019. Twelve formyl phloroglucinol meroterpenoids from the leaves of *Eucalyptus robusta*. *Phytochemistry* 163: 111-117.

Song, Q., Li, X.M., Hu, X.Y., Li, X., Chi, L.P., Li, H.L. & Wang, B.G. 2019. Antibacterial metabolites from Ascidian-derived fungus *Aspergillus clavatus* AS-107. *Phytochemistry Letters* 34: 30-34.

Syukri, D.M., Nwabor, O.F., Singh, S., Ontong, J.C., Wunnoo, S., Paosen, S., Munah, S. & Voravuthikunchai, S.P. 2020. Antibacterial-coated silk surgical sutures by *ex situ* deposition of silver nanoparticles synthesized with *Eucalyptus camaldulensis* eradicates infections. *Journal of Microbiological Methods* 174: 105955.

Tantapakul, C., Promgool, T., Kanokmedhakul, K., Soytong, K., Song, J.J., Hadsadee, S., Jungsuttiwong, S. & Kanokmedhakul, S. 2020. Bioactive xanthoquinodins and epipolythiodioxopiperazines from *Chaetomium globosum* 7s-1, an endophytic fungus isolated from *Rhapis cochinchinensis* (Lour.) Mart. *Natural Product Research* 34(4): 494-502.

Tiwari, A., Singh, P., Jaitley, P., Sharma, S., Prakash, A., Mandil, R., Choudhury, S., Gangwar, N.K. & Garg, S.K. 2018. *Eucalyptus robusta* leaves methanolic extract suppresses inflammatory mediators by specifically targeting TLR4/TLR9, MPO, COX2, iNOS and inflammatory cytokines in experimentally-induced endometritis in rats. *Journal of Ethnopharmacology* 213: 149-158.

Wang, Z.C., Jia, S.T., Cui, J.W., Qu, J.H., Yue, Y.Y., Sun, Q. & Zhang, H.R. 2019. Antioxidant activity of a polysaccharide produced by *Chaetomium globosum* CGMCC 6882. *International Journal of Biological Macromolecules* 141(1): 955-960.

Wang, Y., Arnold, R., Li, G.Q., Xie, Y.J. & Zhou, X.D. 2014. Identification and rapid detection of bacterial wilt in plantation *Eucalyptus* in China. *Australian Forestry* 77(2): 133-139.

Wu, X.L., Wang, S., Liu, C., Zhang, C.F., Guo, J.J. & Shang, X.Y. 2019. A new 2*H*-benzindazole compound from *Alternaria alternata* Shm-1, an endophytic fungus isolated from the fresh wild fruit of *Phellinus igniarius*. *Journal of Natural Medicines* 73(3): 620-626.

Yao, C., Bai, H.H., Zhang, Q., Qian, X.Q., Zhang, X., Wu, L.W., Yang, T. & Li, G.Y. 2019. Secondary metabolites from the fungus *Chaetomium elatum* CIB-412. *Chemistry of Natural Compounds* 55(5): 899-901.

Ye, D.H., Li, T.X., Yi, Y.J., Zhang, X.Z. & Zou, L.K. 2019. Characteristics of endophytic fungi from *Polygonum hydropiper* suggest potential application for P-phytoextraction. *Fungal Ecology* 41: 126-136.

Yuan, T., Zhang, C.Y., Qiu, C.Y., Xia, G.Y., Wang, F., Lin, B., Li, H. & Chen, L.X. 2018. Chemical constituents from *Curcuma longa* L. and their inhibitory effects of nitric oxide production. *Natural Product Research* 32(16): 1887-1892.

Zhao, L., Kim, J.C., Paik, M.J., Lee, W.J. & Hur, J.S. 2017. A multifunctional and possible skin UV protectant, (3*R*)-5-Hydroxymellein, produced by an endolichenic fungus isolated from *Parmotrema austrosinense*. *Molecules* 22(1): 26.

*Corresponding author; email: wskun2001@163.com