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Contributed Paper

Endophytic Fungi from *Artemisia argyi* Levl. et Vant. and Their Bioactivity

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ABSTRACT

A total of 108 isolates of endophytic fungi were isolated from the medicinal plant *Artemisia argyi*. The anti-tumor and anti-microbial bioactivities of the fermentation extracts (FEs) of each isolate were evaluated via different assay methods. Seventeen strains were found to produce bioactive components and identified via ITS rDNA sequence data. The results showed that the FEs from five (4.6 %) of the isolates exhibited anti-tumor activity and fifteen (13.9 %) of the isolates exhibited anti-microbial activity. The phylogenetic tree based on ITS rDNA sequence identified these bioactive isolates into 10 genera in Ascomycetes, and 1 in Basidiomycetes. The main representative group namely Pleosporales had 4 isolates (23.5 %) along with three *Alternaria* spp. The results indicate that the endophytic fungi from *A. argyi* are promising novel source for bioactive compounds.

Keywords: endophytic fungus, *Artemisia argyi*, bioactivity, anti-tumor, anti-microbial

1. INTRODUCTION

Endophytic fungi exist ubiquitously in association with the plant kingdom. Their global biodiversity is enormous, and they are virtually present in almost all plant species studied to date [1, 2]. Since the discovery that an endophytic fungus (*Taxomyces andreanae*) also produced the anti-cancer drug paclitaxel (Taxol®) originally derived from Pacific yew (*Taxus brevifolia*) [3], much attention has being paid to the biodiversity of endophyte, the chemistry and bioactivity of metabolites from endophytic fungi and the relationship between endophytes and their host plants [4-8]. Considering that endophytic fungi have been recognized as potential source of novel natural products for pharmaceutical and agricultural industrial, especially those

secondary metabolites produced by fungal endophytes colonizing medicinal plants [1,9,10], the investigation of bioactivities of the endophytic fungi isolated from traditional medicinal plants has become very important.

As one of the most popular plants in traditional chinese medicine (TCM), the herb *A. argyi* of the Asteraceae family (with a common name Moxa) has been frequently used for treatment of the diseases such as eczema, inflammation, hemostasis, menstruation-related symptoms, and tuberculosis. Recent studies on *A. argyi* found that its extracts exhibited anti-cancer, anti-microbial and anti-feedant activities [11-13]. Several studies have been performed to explore the endophytes of *A. argyi* [14,15], including endophytic actinomycetes and bacteria. To our best knowledge, so far only two fungi of *A. argyi* were investigated [16]. It was reported that the chloroform extracts of *Rhodotorula* sp. and *Fusarium* sp. from *A. argyi* had significant cytotoxic effects (<20 μg/ml) against liver (Hep G2) cancer cell line, with IC_{50} values of 0.9-19.8 μg/ml [16]. Therefore the biodiversity and bioctivity of the endophytic fungi from *A. argyi* is awaiting further research.

Endophytic fungi are often studied at the morphological level and difficult to identify due to failing to sporulate. Molecular phylogenetic analysis based on DNA sequences has been recognized as a reliable method to reveal genetic relationship between the isolates, which could be used to identify and evaluate the isolates at different taxonomic rank [17]. The aim of this study was to isolate and identify endophytic fungi from *A. argyi* via ITS rDNA sequence data and evaluate their anti-tumor and anti-microbial activities against human tumor cell lines and phytopathogenic microorganism for obtaining novel active biomolecules.

2. MATERIALS AND METHODS

2.1 Isolation of Endophytic Fungi

Stem, leaf, root and inflorescences of *A. argyi* were collected from different regions of Guiyang city in Southwest China (including Huaxi, Qingzhen, Kaiyang, Xifeng and Xiuwen counties), during March-November 2012. Plant samples were tagged, stored in a clean plastic bag and taken to the laboratory. The plant specimens were thoroughly washed in running tap water for 10 min before disinfection. The samples were surface-disinfected with 75 % (v/v) ethanol for 1 min followed by 0.1 % (w/v) mercuric chloride for 4 min, subsequently rinsed with sterile water and the outer tissue removed with a sterile scalpel. The leaves were cut into segments (5×5 mm), and stems or roots or inflorescences were cut into pieces (10 mm in length). Surface sterilized plant parts were dried on sterile blotting sheet, chopped, and placed onto the surface of Czapeck agar (CZA) or Potato dextrose agar (PDA) medium in Petri dishes supplemented with 200 U/ml penicillin G and 200 μg/ml streptomycin sulfate for suppressing the growth of bacteria. These plates were incubated at 28°C for 7-15 days. Following incubation, colonies were subsequently re-isolated and placed into PDA and incubated at 28°C for 24-72 h to obtain pure cultures.

2.2 Fermentation and Extraction of Endophytic Fungi

The endophytic fungi were grown under static conditions at 28°C for 30 days in 1,000 ml conical flasks containing Potato dextrose broth (PDB) (300 ml/flask). The fermented whole broth was filtered

through cheesecloth to separate it into supernatant and mycelia. The former was concentrated under reduced pressure to a quarter of the original volume and then extracted three times with ethyl acetate (EtOAc) (150 ml/each time) to give an EtOAc solution, while the latter was extracted three times with EtOAc/water (2:1, v/v) (225 ml/each time). The aqueous and oleophilic phases of extracted EtOAc/water (2:1, v/v) were isolated. All EtOAc solutions were combined and concentrated under reduced pressure to give a fermentation extract (FE).

2.3 Anti-tumor Activity Assay

The determination of anti-tumor activity was carried out based on the MTT assay, which was first described by Mosmann to quantitate mitochondrial dehydrogenase activity [18]. The human tumor cell lines [breast (MCF-7), colon (COLO205) and leukemia (HL-60)] obtained from the Cell Line Bank of the Chinese Academy of Science were used in the present study. The cell lines were grown in RPMI-1640 supplemented with 10 % fetal bovine serum under a humidified atmosphere of 5 % CO_2 and 95 % air at 37°C. An aliquot (200 µl) of these cell suspensions at a density of 3×10^4 cell/ml was plated in 96-well microtiter plates and incubated for 24 h at the above conditions. Then 2 µl of the FE dimethyl sulfoxide (DMSO) solutions at different concentrations was added to each well and further incubated for 72 h in the same conditions. MTT solution (20 µl of 5 mg/ml in IPMI-1640 medium) was added to each well and incubated for 4 h. Old medium containing MTT (150 µl) was then gently replaced by DMSO and pipetted to dissolve any formazan crystals that had formed. Absorbance was then determined on a Spectra Max Plus plate reader at 570 nm. Growth inhibition rate was calculated as $(OD_{control} - OD_{treated})/OD_{control} \times 100$.

2.4 Anti-bacterial Activity Assay

The agar-well diffusion method was applied to evaluate the anti-bacterial activity [19]. The tested pathogenic bacteria were *Clavbacter michiganensis* and *Pseudomonas solanacearum* obtained from Plant Protection and Quarantine Station of Guiyang in China. Wells with the diameter of 7 mm were made in agar and 100 µl of the FE solutions (in EtOAc) at 200 µg/ml was pipetted into the well. The plates were incubated at 30°C for 24-48 h and the clear zones of inhibition around the fungal extracts were measured and compared with negative control.

2.5 Anti-fungal Activity Assay

The anti-fungal activity of the FE was determined on phytopathogenic fungi: *Fusarium graminearum*, *Rhizoctorzia solani* and *Phytophthora capsici* obtained from Plant Protection and Quarantine Station of Guiyang in China. A 7-mm diameter mycelial disk cut from the edge of an actively growing culture (3 days old) of the phytopathogenic fungi was placed in the center of a 9-cm diameter Petri dish containing PDA plus each FE. The concentration of each FE in PDA was 1 mg/ml. After 3 days at 28°C, the colony diameter of each strain was measured with the original mycelial disk diameter (5 mm) subtracted from this measurement. Percentage inhibition was calculated as $(1 - a/b)\times100$, where a was the colony diameter in Petri dishes with FE and b was the mean colony diameter in Petri dishes without FE.

2.6 Molecular Identification of Endophytic Fungi

The fungal taxa used in this study were listed in Table 1. Total genomic DNA of the endophytic fungi was extracted directly from actively growing mycelium scraped from PDA plates, using the Fungal gDNA Kit GD2416 (Biomiga, CA, USA) in accordance with manufacturers' protocol. Nuclear ITS rDNA was amplified with primer pairs ITS1 (TCC GTA GGT GAA CCT GCG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC). Amplification reactions were performed in a Bio-RAD PTC-200 thermocycler, in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1× PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 U of Taq polymerase. PCR amplification products were separated by 1 % (w/v) agarose gels and stained with ethidium bromide (0.5 µg/ml) for visual examination. Sequencing was performed with an ABI PRISM 3730 DNA autosequencer using either dRhodamine terminator or Big Dye Terminator (Applied Biosystems Inc., Foster 19 City, California).

Sequences generated for this study were aligned with additional sequences downloaded from GenBank using BioEdit [20] and ClustalX [21]. Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps manually adjusted to optimize alignment. The tree construction procedure was performed in PAUP* version 4.0b10 [22]. All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with tree bisection reconnection (TBR) branch swapping and 1,000 random sequence additions. Max-trees were set to 5,000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1,000 replicates [23]. Descriptive tree statistics including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated.

3. RESULTS

One hundred and eight endophytic fungal strains were isolated from leaf, stem, root and inflorescence of *A. argyi*. All the isolates were screened for their anti-tumor and anti-phytopathogenic microbial potential. Seventeen endophytic fungal isolates that showed bioactivity were taken up for molecular identification. The phylogenetic tree was constructed with ITS rDNA sequences from this study along with their allies from Genbank (Figure 1). The tree showed that the isolates belonging to diverse fungal group were mainly distributed within Ascomycetes, while only one belonged to Basidiomycetes (Sporidiobolales). Ten different fungal groups were identified, of which nine belonged to Pleosporales, Capnodiales, Eurotiales, Diaporthales, Hypocreales, Phyllachorales, Sordariomycetidae (subclass), Sordariales and Xylariales in Ascomycetes. Most groups were supported by high bootstrap values. The main representative group was Pleosporales with 4 isolates (23.5 %), which were integrated by three *Alternaria* species. The groups Diaporthales, Hypocreales, Sordariales and Xylariales included two taxa, and the groups Capnodiales, Eurotiales, Phyllacho-rales, Sordariomycetidae (subclass) and Sporidiobolales included only one taxon (Figure 1 and Table 1).

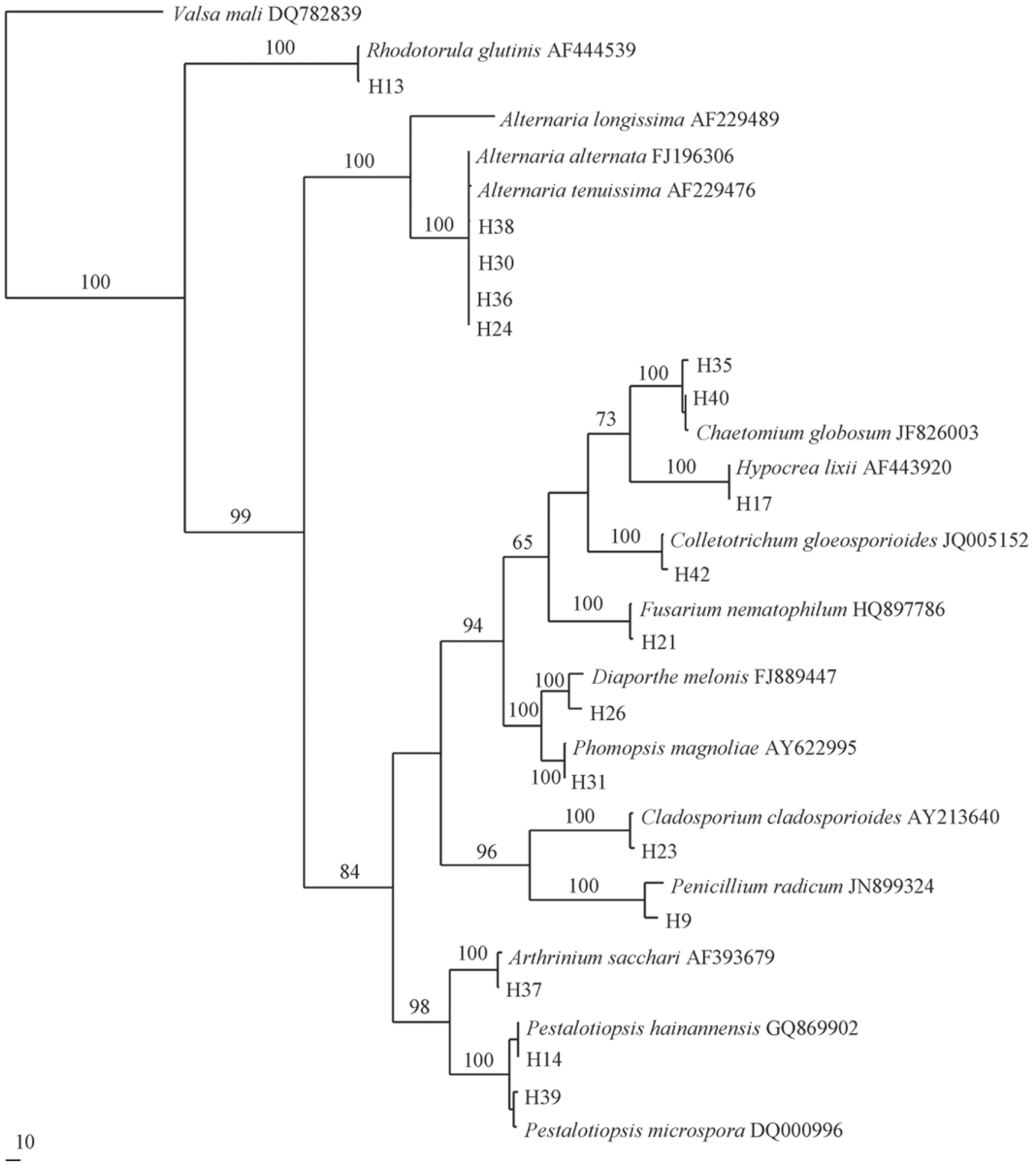

Figure 1. Phylogenetic tree showing the relationship between endophytic fungi of *A. argyi* and other related fungal species. The tree was constructed based on ITS rDNA sequence using MP analysis. Bootstrap support values above 50% are shown. The tree is rooted with *Valsa mali*.

Table 1. In vitro anti-phytopathogenic microbial activities of the endophytic fungi of this study.

Isolate	Gene bank accession number	Taxa	Diameter of inhibition zone (cm) Plant pathogenic bacteria Conc. 200 µg/ml		Growth inhibition (%) Plant pathogenic fungi Conc. 1 mg/ml		
			C. michiganensis	*P. solanacearum*	*F. graminearum*	*R. solani*	*P. capsici*
H38	KF053668	*Alternaria* sp.	–[a]	–	15.15 ± 3.03	–	–
H24	KF053667		–	1.10 ± 0.10	–	–	–
H36	KF053678		1.47 ± 0.10	–	–	–	–
H30	KF053670		–	–	–	27.48 ± 1.56	–
H37	KF053669	*Arthrinium* sp.	–	–	–	–	11.11 ± 3.70
H40	KF053679	*Chaetomium* sp.	–	1.20 ± 0.10	–	–	–
H35	KF053680		–	–	12.12 ± 3.03	–	–
H23	KF053671	*Cladosporium* sp.	–	–	–	–	23.46 ± .66
H42	KF053677	*Colletotrichum* sp.	–	–	–	–	–
H21	KF053676	*Fusarium* sp.	–	–	–	–	12.35 ± 7.71
H9	KF053672	*Penicillium* sp.	–	1.00 ± 0.20	–	15.77 ± 3.40	–
H14	KF053681	*Pestalotiopsis* sp.	2.87 ± 0.10	–	11.11 ± 6.31	–	–
H39	KF053682		–	–	–	13.51 ± 6.19	–
H31	KF053674	*Phomopsis* sp.	–	–	–	–	34.57 ± 2.14
H26	KF053673	*Diaporthe* sp.	1.33 ± 0.20	–	–	–	–
H13	KF053683	*Rhodotorula* sp.	–	–	–	–	–
H17	KF053675	*Trichoderma* sp.	1.13 ± 0.10	–	–	–	–

[a] No inhibition

Table 2. In vitro anti-tumor activities of the endophytic fungi of this study.

Isolate	Gene bank accession number	Taxa	Growth inhibition (%) Human tumor cell lines Conc. 20 μg/ml (200 μg/ml)		
			Breast MCF-7	Colon COLO-205	Leukemia HL-60
H38	KF053668		54.16 ± 1.07 (83.76 ± 2.56)	16.41 ± 7.74 (96.21 ± 0.17)	48.86 ± 13.07 (49.75 ± 9.27)
H24	KF053667	*Alternaria* sp.	–[a]	–	–
H36	KF053678		–	–	–
H30	KF053670		–	–	–
H37	KF053669	*Arthrinium* sp.	–	–	–
H40	KF053679	*Chaetomium* sp.	–	–	–
H35	KF053680		–	–	–
H23	KF053671	*Cladosporium* sp.	–	–	–
H42	KF053677	*Colletotrichum* sp.	26.95 ± 2.44 (49.53 ± 0.96)	71.99 ± 1.11 (76.27 ± 6.58)	11.22 ± 3.18 (35.82 ± 3.01)
H21	KF053676	*Fusarium* sp.	–	–	–
H9	KF053672	*Penicillium* sp.	–	–	–
H14	KF053681	*Pestalotiopsis* sp.	71.43 ± 1.96 (93.51 ± 2.94)	53.47 ± 1.04 (95.98 ± 0.68)	97.03 ± 0.7 4 (96.96 ± 1.53)
H39	KF053682		–	–	–
H31	KF053674	*Phomopsis* sp.	0.00 ± 0.00 (27.66 ± 0.66)	0.00 ± 0.00 (41.79 ± 0.86)	81.69 ± 0.72 (81.92 ± 0.33)
H26	KF053673	*Diaporthe* sp.	–	–	–
H13	KF053683	*Rhodotorula* sp.	23.56 ± 8.39 (89.69 ± 3.31)	16.41 ± 7.75 (96.21 ± 0.73)	78.28 ± 2.11 (93.28 ± 1.66)
H17	KF053675	*Trichoderma* sp.	–	–	–

[a] No inhibition

The results in Table 1, Table 2 and Figure 2 showed that endophytic fungi in this study exhibited anti-microbial and anti-tumor activity. The FEs of H9 and H14 exhibited activity against bacterial and fungi. Especially, the FE of H14 showed strong activity against *Clavbacter michiganensis*. On the other hand isolate H30 produced anti-fungal activity against *Rhizoctorzia solani*, whereas the FEs from H31 and H23 showed anti-fungal activity against *Phytophthora capsici*. Five isolates from five orders (Pleosporales, Phyllachorales, Xylariales, Diaporthales and Sporidiobolales) exhibited anti-tumor activity and were active against different tumor cell lines. The FE from H14 (*Pestalotiopsis hainannensis*) at a concentration of 20 μg/ml was active against all tested cell lines with 71.42, 53.47 and 97.03 % inhibition of MCF-7 (breast), COLO205 (colon) and HL-60 (leukemia) cell lines respectively. The FEs from H31 (*Phomopsis mail*) and H13 (*Rhodotorula glutinis*) showed 81.69 and 78.28% inhibition of HL-60 leukemia cell line at a concentration

of 20 µg/ml . The FE from H38 (*Alternaria* sp.) was active only against MCF-7 breast cell line with 54.16 % inhibition. Similarly, isolate H42 (*Colletotrichum gloeosporioides*) was active only against COLO205 colon cell line with 71.99 % inhibition at the same concentration. In conclusion, the FE from H14 showed broad spectrum anti-tumor and anti-microbial activity, whereas the FEs from H42 and H13 showed anti-tumor activities only against COLO205 (colon) and HL-60 (leukemia) cell lines, respectively. On the other hand the FE from H31 showed strong activity only against *P. capsici* and HL-60 (leukemia) cell line.

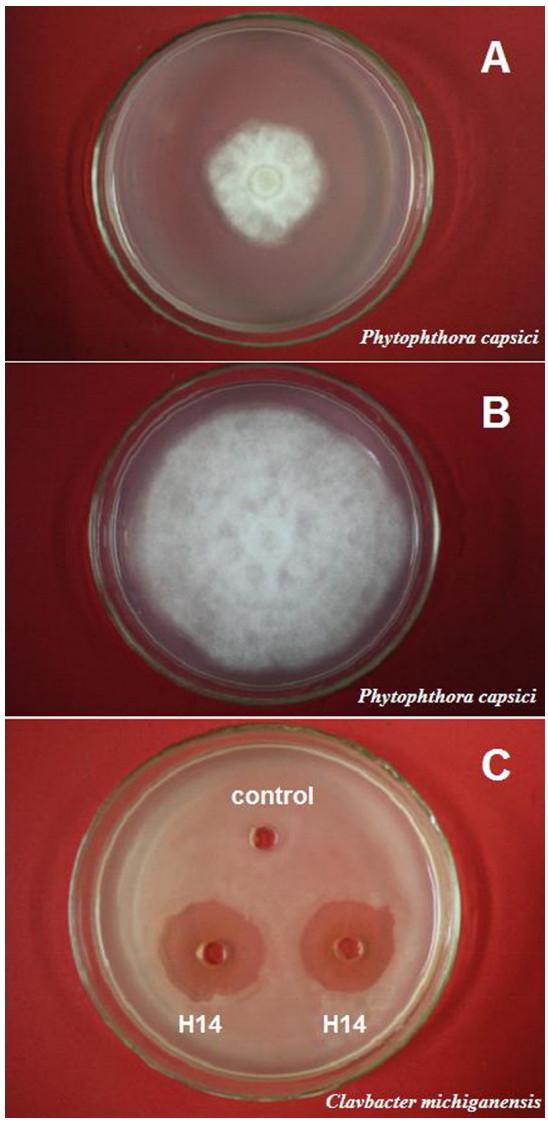

Figure 2. Anti-phytopathogenic microbial activities. A. Growth inhibition of *P. capsici* by EtOAc FE (1 mg/ml) of H31 (*Phomopsis magnoliae*) isolated from *A. argyi*; B. Control, EtOAc (1 mg/ml); C. Inhibition zone of *C. michiganensis* by EtOAc FE (200 µg/ml) of H14 (*Pestalotiopsis hainannensis*) isolated from *A. argyi.*

4. DISCUSSION

Endophytic fungi represent an underexplored source of novel compounds. The discoveries of biologically active fungal metabolites including new antibiotics, chemothereputic agents and agrochemicals have become a focus of the scientific community worldwide. These fungal metabolites are generally recognized as highly effective, possess low toxicity, and have a minor environmental impact [1, 24-26]. In previous studies, the endophytes from only *A. annua* of *Artemisia* genus were reported [27-33]. A number of bioactive constituents were isolated from the endophytes including *Colletotrichum* sp., *Hypoxylon truncatum* and *Myrothecium roridum* in association with *A. annua*, [27,30,31].

In this study, we have investigated endophytic fungi associated with *A. argyi* at the counties around Guiyang city in southwest China, of which 17 isolates displayed remarkable anti-tumor and anti-microbial activities. *Alternaria* sp. and *Pestalotiopsis* sp. from different parts of the same host plant respectively showed variation in their metabolites production capabilities. The bioactive endophytic fungi associated with *A. argyi* comprised a number of cosmopolitan species such as *Alternaria* sp., *Colletotrichum* sp., *Phomopsis* sp. and *Pestalotiopsis* sp. All these genera have been previously isolated as endophytes, not only in other *Artemisia* species [27-33], but also in a wide range of host plant in the tropics [34-36]. However, all these genera hosted in *A. argyi* are reported for the first time in this study except *Rhodotorula* sp. and *Fusarium* sp., which indicate that *A. argyi* harbours diverse mycota.

Traditional approaches to identify fungal endophytes involve microscopic analysis of morphological characteristics. However, significant portions of endophytic isolates consist of sterile mycelia and consequently can not be identified via these approaches. Phylogenetic analysis of rDNA sequences have been successfully employed for the identification of different morphospecies [37-42]. In the present study, each bioactive isolate was cultured, categorized by morphology and identified via phylogenetic analysis of ITS rDNA sequences.

Our studies suggest that the members of Ascomycetes showed high bioactive versatility. The members of this group produced a variety of bioactive components including anti-phytopathogenic microbial agents active against *Clavbacter michiganensis* and *Phytophthora capsici*, in addition to bioactivity against three tumor cell lines. Further research is awaiting for understanding the chemical nature of the biomolecules produced by members of Ascomycetes and their anti-tumor and anti-phytopathogenic microbial activities.

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REFERENCES

[1] Strobel G.A. and Daisy B., Bioprospecting for microbial endophytes and their natural products, *Microbiol. Mol. Biol. Rev.*, 2003; **67(4):** 491-502.

[2] Huang W.Y., Cai Y.Z., Surveswaran S., Hyde K.D., Corke H. and Sun M., Molecular phylogenetic identification of endophytic fungi isolated from three *Artemisia* species, *Fungal Divers.*, 2009; **36:** 69-88.

[3] Stierle A., Strobel G., Stierle D., Grothaus P. and Bignami G., The search for a taxol-producing microorganism among the endophytic fungi of the pacific yew, *Taxus brevifolia*, *J. Nat. Prod.*, 1995; **58:** 1315-1324.

[4] Tan R.X. and Zou W.X., Endophytes: A rich source of functional metabolites, *Nat. Prod. Rep.*, 2001; **18:** 448-459.

[5] Schulz B., Boyle C., Draeger S. and Rmmert A.K., Endophytic fungi: A source of novel biologically active secondary metabolites, *Mycol. Res.*, 2002; **106:** 996-1004.

[6] Kumar D.S.S., Lau C.S., Wan J.M.F., Yang D. and Hyde K.D., Immunomodulatory compounds from Pestalotiopsis leucoths (HKUCC 10197), an endophytic fungus of *Tripterygium wilfordii*, *Life Sci.*, 2005; **78:** 147-156.

[7] Rungjindamai N., Pinruan U., Choeyklin R., Hattori T. and Jones E.B.G., Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, *Elaeis guineensis*, in Thailand, *Fungal Divers.*, 2008; **33:** 139-162.

[8] Tao G., Liu Z.Y., Hyde K.D., Lui X.Z. and Yu Z.N., Whole rDNA analysis reveals novel and endophytic fungi in *Bletilla ochracea* (Orchidaceae), *Fungal Divers.*, 2008; **33:** 101-122.

[9] Hyde K.D. and Soytong K., The fungal endophyte dilemma, *Fungal Divers.*, 2008; **33:** 163-173.

[10] Mitchell A.M., Strobel G.A., Hess W.M., Vargas P.N. and Ezra D., Muscodor crispans, a novel endophyte from *Ananas ananassoides* in the Bolivian Amazon, *Fungal Divers.*, 2008; **31:** 37-43.

[11] Lan M.B., Zhang Y.H., Zheng Y., Yuan H.H., Zhao H.L. and Gao F., Antioxidant and immunomodulatory activities of polysaccharides from Moxa (*Artemisia argyi*) Leaf, *Food Sci. Biotechnol.*, 2010; **19(6):** 1463-1469.

[12] Hu L., Cui C.X., Wu Y.B. and Pei Y.G., Research advances on chemical constituents and bioactivities of *Artemisia argyi*, *J. Henan Inst. Sci. Technol.*, 2010; **38(4):** 75-78.

[13] Guo Y., Pan Y.M., Gao Z.M., Wang K., Peng M. and Cao S., Inhibitive activity of the extracts of plants in *Artemisia* against *Fusarium oxysporum* f. sp. *vasinfectum* and *Fusarium momiliforme*, *Agricultural Sci. Technology*, 2010; **11:** 128-131, 154.

[14] Zhang X.M., Zhang L.P. and Li J.C., Isolation, phylogenetic analysis and relative characteristics of endophytic actinomycete isolated from *Artemisiae argyi*, *J. Agricultural University of Hebei*, 2011; **34(6):** 60-64, 82.

[15] Xu Y.J., Zhao L.F., Chen P., Jiang X.R. and Wei G.H., Isolation, screening and characterization of phytopathogen antagonistic endophytes from wild *Artemisia argyi*, *Acta Ecologica. Sinica*, 2013; **33(12):** 3697-3705.

[16] Hsieh P.W., Hsu L.C., Lai C.H., Wu C.C., Hwang T.L., Lin Y.K. and Wu Y.C., Evaluation of the bioactivities of extracts of endophytes isolated from Taiwanese herbal plants, *World J.*

Microbiol. Biotechnol., 2009; **25:** 1461-1469.

[17] Bruns T.D., Vilgalys R., Barns S.M., Gonzalez D., Hibbett D.S., Lane D.J., Simon L., Stickel S., Szaro T.M., Weisburg W.G. and Sogin M.L., Evolutionary relationships within the fungi: Analyses of nuclear small subunit rRNA sequences, *Mol. Phylogenet. Evol.*, 1992; **1:** 231-241.

[18] Mosmann T., Rapid calorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay, *J. Immunol. Methods*, 1983; **65:** 55-63.

[19] Gomez S., Cosson C. and Deschamps A.M., Evidence for a bacteriocinlike substance produced by a new strain of *Streptococcus* sp., inhibitory to Gram-positive food-borne pathogens, *Res. Microbiol.*, 1997; **148:** 757-766.

[20] Hall T.A., Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, *Nucleic Acids Symp.*, 1999; **41:** 95-98.

[21] Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G., The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Res.*, 1997; **24:** 4876-4882.

[22] Swofford D.L., PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4.0 Sinauer Associates, Sunderland, MA, 2004.

[23] Felsenstein J., Confidence intervals on phylogenetics: An approach using bootstrap, *Evolution*, 1985; **39:** 783-791.

[24] Pearce C., Biologically active fungal metabolites, *Adv. Appl. Microbiol.*, 1997; **44**:1-80.

[25] Smith S.A., Tank D.C., Boulanger L.A., Bascom-Slack C.A., Eisenman K., Kingery D., Babbs B., Fenn K., Greene J.S., Hann B.D., Keehner J., Kelley-Swift E.G., Kembaiyan V., Lee S.J., Li P., Light D.Y., Lin E.H., Ma C., Moore E., Schorn M.A., Vekhter D., Nunez P.V., Strobel G.A., Donoghue M.J. and Strobel S.A., Bioactive endophytes warrants intensified exploration and conservation, *PLoS One*, 2008; **3:** 30-52.

[26] Xu J., Ebada S.S. and Proksch P., Pestalotiopsis a highly creative genus: Chemistry and bioactivity of secondary metabolites, *Fungal Divers.*, 2010; **44:** 15-31.

[27] Lu H., Zou W.X., Meng J.C., Hu J. and Tan R.X., New bioactive metabolites produced by *Colletotrichum* sp., an endophytic fungus in *Artemisia annua*., *Plant Sci.*, 2000; **151:** 67-73.

[28] Zou W.X., Meng J.C., Lu H., Chen G.X., Shi G.X., Zhang T.Y. and Tan R.X., Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*, *J. Nat. Prod.*, 2000; **63:** 1529-1530.

[29] Liu C.H., Zou W.X., Lu H. and Tan R.X., Antifungal activity of *Artemisia annua* endophyte cultures against phyto-pathogenic fungi, *J. Biotechnol.*, 2001; **88:** 277-282.

[30] Gu W., Ge H.M., Song Y.C., Ding H., Zhu H.L., Zhao X.A. and Tan R.X., Cytotoxic Benzo[j] fluoranthene metabolites from Hypoxylon truncatum IFB-18, an endophyte of *Artemisia annua*, *J. Nat. Prod.*, 2007; **70:** 114-117.

[31] Shen L., Shi D.H., Song Y.C. and Tan R.X., Chemical constituents of liquid culture of endophyte IFB-E012 in *Artemisia annua*, *Chin. J. Nat. Med.*, 2009; **7(5):** 354-356.

[32] Li J., Zhao G.Z., Huang H.Y., Qin S., Zhou W.Y., Zhao L.X., Xu L.H.,

Zhang S., Li W.J. and Strobel G., Isolation and characterization of culturable endophytic actinobacteria associated with *Artemisia annua* L., *Antonie van Leeuwenhoek*, 2012; **101(3):** 515-527.

[33] Zhang H.W., Bai X.L., Wu B.X., Evaluation of antimicrobial activies of extracts of endophytic fungi from *Artemisia annua*, *Bangladesh J. Pharmacol.*, 2012; **7:** 120-123.

[34] Azevedo J.L., Macchroni W., Pereira J.O. and Araújo W.L., Endophytic microorganisms: A review on insect control and recent advances on tropical plants, *Bio.-Technol.*, 2000; **3:** 40-65.

[35] Huang W.Y., Cai Y.Z., Hyde K.D., Corke H. and Sun M., Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants, *Fungal Divers.*, 2008; **33:** 61-75.

[36] Prihastuti H., Cai L., Chen H., McKenzie E.H.C. and Hyde K.D., Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. *Fungal Divers.*, 2009; **39:** 89-109.

[37] Guo L.D., Hyde K.D. and Liew E.C.Y., Detection and identification of endophytic fungi within frond tissues of *Livistona chinensis* based on rDNA sequence, *Mol. Phylogenet. Evol.*, 2001; **20:** 1-13.

[38] Guo L.D., Huang G.R., Wang Y., He W.H., Zheng W.H. and Hyde K.D., Molecular identification of white morphotype strains of endophytic fungi from *Pinus tabulaeformis*, *Mycol. Res.*, 2003; **107:** 680-688.

[39] Promputtha L., Jeewon R., Lumyong S., Mckenzie E.H.C. and Hyde K.D., Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from *Magnolia liliifera* (Magnoliaceae), *Fungal Divers.*, 2005; **20:** 167-186.

[40] Wang Y., Guo L.D. and Hyde K.D., Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences, *Fungal Divers.*, 2005); **20:** 235-260.

[41] Wang Y.T., Lo H.S. and Wang P.H., Endophytic fungi from *Taxus mairei* in Taiwan: first report of *Colletotrichum gloeosporioides* as an endophyte of *Taxus mairei*, *Bot. Stud.*, 2008; **49:** 39-43.

[42] Liu K., Ding X., Deng B. and Chen W., Isolation and characterization of endophytic taxol-producing fungi from *Taxus chinensis*, *J. Ind. Microbiol. Biotech.*, 2009; **36:** 1171-1177.