

<b>Dissertation Title</b>	Fungal Endophytes from <i>Dendrobium</i>
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## **ABSTRACT**

*Dendrobium*, as one of the largest genera in Orchidaceae, is spread in Atlantic Islands and Asia. They are well known for commercial, horticultural, medicinal, and ornamental values. For decades, the general compositions, function, and secondary metabolites of culturable fungal endophytes of *Dendrobium* species have been investigated. However, due to the lacking morphological characteristics and ambiguous phylogenetic analysis resulting from the single gene ITS amplification for most research, the species composition of fungal endophytes in *Dendrobium* remains unclear. Furthermore, the species resolution for the taxonomy of fungal endophytes contributes to discovering more cryptic taxa and providing further information for fungal resources. This study aims at identifying fungal endophytes with morpho-molecular methods to give a general conclusion for fungal endophytes composition of 65 *Dendrobium* individuals (Including 25 known species and other repeat or not identified species) in southwestern China and northern Thailand. Meanwhile, the cytotoxic activities screening of selected fungal endophytes of *Dendrobium* species were carried out in this study.

To achieve a natural classification, the endophytic species resolution was implemented by sporulation with various media including CMA, MEA, OA, PDA, SDA, SNA, WA (with toothpicks/sample debris/slide culture/pine needle) and multi-gene phylogenetic analysis with partial genes of ACT, ITS, CAL, GAPDH, HIS,

LSU, RPB1, RPB2, SSU, TEF -1 $\alpha$ , TUB2. A total of 750 culturable fungal endophytic strains were isolated from 65 *Dendrobium* samples and identified to 110 species with 25 novel taxa and 50 new host records were identified. These endophytic species are distributed in 70 genera of 52 families belonging to 24 orders of 8 classes mainly in Ascomycota (94.6%) and less in Basidiomycota (5.4%). In Sordariomycetes, 20 novel species (8 in *Colletotrichum*, 3 in *Xylaria*, 2 in *Diaporthe*, 2 in *Fusarium*, 2 in *Hypoxyton*, 1 in *Annulohypoxyton*, 1 in *Neopestalotiopsis*, 1 in *Nemania*) and 40 new host records were reported. Four and one novel species were discovered from Dothideomycetes (*Aureobasidium*, *Epicoccum*, *Mycosphaerella*, *Phyllosticta*) and Leotiomycetes (*Pezicula*), respectively. The rest isolates were categorized to Agaricomycetes (3.4%), Eurotiomycetes (3.2%), Pezizomycetes (1.4%), Saccharomycetes (5.2%) and Ustilaginomycetes (0.1%). *Colletotrichum*, *Diaporthe* and *Fusarium* were the three frequently isolated genera in Sordariomycetes also in this phylum. *Colletotrichum siamense* is the most frequently isolated species. *Phyllosticta* species are the most isolated group in Dothideomycetes. *Meyerozyma* and *Penicillium* take the majority of Saccharomycetes and Eurotiomycetes, respectively. In Basidiomycota, 13 genera were discovered in Agaricomycetes. *Pseudozyma tsukubaensis* is the solo species identified in Ustilaginomycetes. The mycorrhizal fungi *Tulasnella* sp. represented by several strains were isolated from roots but grow extremely slowly and died very soon.

In this study, there is no universal media or cultivation method for all endophytes sporulation. The whole sporulation rate is 5%. The cultivation with sterilized host debris or pine needles failed to improve the sporulation rates. The media with low concentrations were recommended for enhancing the sporulation.

The highest endophyte isolation rate 31.3% is from *Dendrobium* stems followed by 30.9% of leaves. The separation rates of roots and flowers are 28.4% and 9.2% respectively. Most species identified in this study are phylogenetically nested

within known pathogens that have been reported from other plant hosts including some orchids. The host, geographic and organ specificity between fungal endophytes and *Dendrobium* species were rarely observed. Only the well-known leaf inhabiting endophyte *Phyllosticta capitalensis* was mostly separated from the *Dendrobium* leaves.

In this study, cytotoxic assays were carried out with 153 crude extracts including some further fractions. The results showed that 10 species possess cytotoxic potentials that vary from medium to strong degrees. Two newly introduced species in this study—*Colletotrichum doitungense* and *Neopestalotiopsis dendrobii* performed well in inhibiting K562 and PC3 growth. The crude extracts of *Hypoxylon investiens* (MFLUCC 15-1188), *Neopestalotiopsis dendrobii* (MFLUCC 14-0106) and *Xylaria venosula* (MFLUCC 21-0013) performed the highest inhibitory rates towards A549 (64.8%), K562 (89%) and PC3 (55.8%) cell lines, respectively. Several endophytic xylarialean strains stand out of other species which perhaps indicate that they contain natural compounds for inhibiting tumor cell growth.

This study firstly provides a fungal endophytic species list, which supplies a further knowledge of the diversity of fungal endophytes associated with *Dendrobium* species. Meanwhile, the taxonomy of fungal endophytes showed some cryptic taxa and facilitate phylogenetic stability. Therefore, they should have a place in the fungal taxonomic world. However, to give strong evidence, more type or epitype specimens of fungal endophyte with more protein gene annotation data are expected in future work. With interdisciplinary exploration, we may break through the dilemma caused by method limitations and be free from the laborious and low effective endophyte identification work. The results also indicate these fungal assemblages can be a promising repertoire to produce interesting bioactive compounds.

**Keywords:** Ascomycotina, Bioactivity, Biodiversity, Checklist, Endophytic, Multi-gene, Orchids, Phylogeny, Taxonomy

