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## Isolation of Endophytic Fungi from Some Orchid Varieties

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Sour, V.\* , Phonpho, S. and Soyong, K.

Faculty of Agricultural Technoloty, King Mungkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

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**Abstract** Endophytic fungi from orchids in Chantaburi province was studied. Endophytic fungi were isolated from 9 orchid varieties in greenhouse condition. Leaves and roots of *Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobium hercoglossum*, *Dendrobium palpebrae*, *Torenia fournieri*, *Doritis pulcherrima*, *Dendrobium crumenatum*, *Dendrobium friedericksianum* and *Grammatophyllum specinocum* were collected. All samples were collected then brought to laboratory. Isolation was done by surface sterilization, removed the outer surface and got inner tissues then place on water agar and transferred culture to potato dextrose agar to be pure culture. The morphological identification based on colony and agar color, liquid drops, colony shapes, the growth rate and spores characteristic Twelve species are identified as *Chaetomium cochliodes*, *Chaetomium cupreum*, *Xylaria* sp., *Colletotrichum* sp., *Nigrospora* sp., *Phoma* sp. *Curvularia* sp., *Fusarium* sp., *Pestalotiopsis* sp., *Corynascus* sp. and *Cladosporium*-like fungi.

**Keywords:** Endophytic fungi, Orchids

### Introduction

Plants are always colonized with other microorganisms. Endophytic fungi are one kind of microorganism that live within healthy plant tissue causing no harm to the host plant. The meaning of endophytic fungi is so broad. Modern usage of the term endophytic fungi in mycology refers to those fungi which live within leaves stems roots and other part of apparently healthy host plants and can not be seen in visually signs of infection (Stone *et al.*, 2004). Dreyfuss and Chapela (1994) estimated that there may be at least one million species of endophytic fungi alone. It means that almost all plant species are usually infected with endophytic fungi.

Nowaday endophytic fungi take attention from many scientists as estimated that such species may useful as sources of anticancer, antidiabetic, insecticidal and immunosuppressive compounds (Strobel and Daisy, 2003). Endophytic fungi from medicinal plants can be used for the development of drugs. For

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\* **Coressponding author:** Sour, V.; **Email:** [sourvannak@hotmail.com](mailto:sourvannak@hotmail.com)

example *Fusarium oxysporum* isolated from *Catharanthus roseus* plant can produce Vinblastine and Vincristine, excellent anti-cancer drugs (Kumar *et al.*, 2013).

Orchids are monocotyledonous plants and taxonomically belong to Orchidales and Orchidaceae. There are 15000 to 25000 of orchid species distributed all around the world, mostly can found in tropical environment. (Thamasiri, 2005). Fungal endophytes have now been investigated in a large number of orchid species from around the world (Perkins *et al.*, 1995). The fungi involved with orchids are almost all members of the phylum Basidiomycota group. The results may vary with the species of orchid.

## **Materials and methods**

### ***Plant sample collection***

Roots and leaves of 9 orchid varieties (*Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobium hercoglossum*, *Dendrobium palpebrae*, *Torenia fournieri*, *Doritis pulcherrima*, *Dendrobium crumenatum*, *Dendrobium friedricksianum* and *Grammatophyllum specinocum*). Sample were collected in greenhouse from Rajamangala University Technology Tawan-ok Chantaburi Campus, Chantaburi province, Thailand on 1<sup>st</sup> December 2014.

All samples were placed in sterile polythene bags and brought to laboratory in an icebox and stored at 4.5 °C before isolating endophytic fungi within 48 hours.

### ***Isolation and identification of endophytic fungi***

The surface sterilization was done by procedure used by Blumenstein (2010) with some modifications. Symptomless roots of each plant species were cut into 2–3 mm sections were rinsed in a sequence distilled water for 1 min, 75% ethanol for 30s, 3% NaClO for 1 min, and 75% ethanol for 30s and then rinsed in sterile distilled water three times. Each root was placed on water agar (WA). The isolates were subcultured on potato dextrose agar for morphological identification and conidial production.

The endophytic fungi were identified initially according to their colony, mycelium and spore characteristics according to the discription of Barnett and Hunter (1998) and Von Arx (1978).

## Results and Discussion

Many isolates can't identify the species because it doesn't produce spores. Twelve species belonging to Ascomycota were isolated from 9 orchid varieties. They are as follow:

### 1. *Chaetomium cochliodes*

Habitat: *Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobium hercoglossum* and *Torenia fournieri*.

The colonies are quit slowly growing, for cultured on PDA medium in room temperature for 7 days have 7 cm in diameter, cottony and white in color initially and then become soft-green. *C. cochliodes* has distinctive small brown 'lemon' or 'football' shaped ascospores. (Arx J.A. von *et al.*, 1986).

This species also isolated as endophytic fungi from *Cirsium arvense*, (Gange *et al.*, 2012). Li Gy *et al.* (2006) indicated that epipolythiodioxopiperazines and chaetocochin were isolated from the ethyl acetate extract of the solid-state fermented rice culture of the fungus *Chaetomium cochliodes*. Their structures were elucidated on the basis of spectroscopic analysis. Compounds 1, 3, and 4 exhibited significant cytotoxicity in vitro against cancer cell lines Bre-04, Lu-04, and N-04,



**Figure 1.** *Chaetomium cochliodes* A: The colony that culture on PDA 30 days, B The spores under microscops 10X C The ascospores under microscops 40X

### 2. *Chaetomium cupreum*

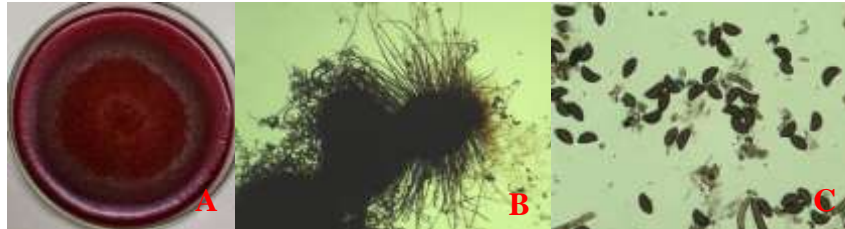
Habitat: *Grammatophyllum scriptum*.

The growth was quit slow, cultured on PDA medium for 7 days the growth have 4.4 cm in diameter. The hypheae are red and it produces the red color around mycelia.

Septate hyphae, perithecia, asci and ascospores are visualized. Perithecia are large, red color, fragile, globose to flask shaped and have filamentous, hair-like, brown to black appendages (setae) on their surface. Perithecia have

ostioles (small rounded openings) and contain asci and ascospores inside (De Hoog *et al.*, 2000).

This species was isolated as endophytic fungus from *Macleaya cordata* and have ability to antifungal activity against *Rhizoctonia solani*, *Botrytis cinerea*, *Pytium ultimum*, and antitumor activities against HL-60 and A549. It is toxic to *Artemia salina* (Bi *et al.*, 2010).



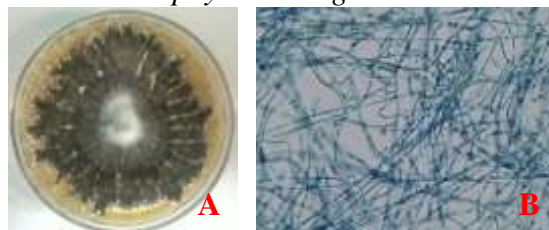
**Figure 2.** *Chaetomium cupreum* **A:** The colony cultured on PDA 30 days **B:** The spores under microscops 10X and **C:**The ascospores under microscops 40X

### 3. *Xylaria* sp.

Habitate: *Cymbidium dayanum*, *Dendrobium hercoglossum*, *Doritis pulcherrima*, *Dendrobium crumenatum*, *Dendrobium friedericksianum* and *Grammatophyllum specinocum*.

The fungi have white mycelia; the growth was quit slowl, for cultured on PDA in room temperature for 7 days have 5.8 cm in diameter. They produce the stroma on 20<sup>th</sup> day but didn't produce ascus and ascospore.

*Xylaria* sp. was the common species that can isolated from other orchid varieties. Previous research also found that this species are most colonized in 7 *Dendrombium* sp. in China (Chen *et al.* 2013). Sawmya *et al.* (2013) also isolated *Xylaria* sp. from *Bulbophyllum neilgherrense* and *Pholidota pallida*.



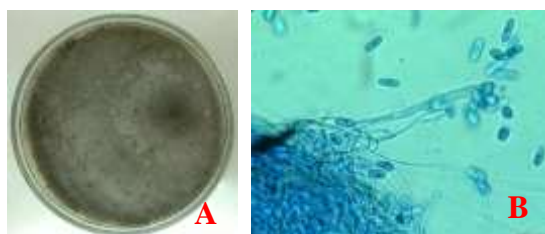
**Figure 3.** *Xylaria* sp. **A:** Colony culture on PDA 30 days **B:** Mycelia of xylaria sp. under microscops 40X

#### 4. *Colletotrichum* sp.

Habitats: *Cymbidium dayanum*, *Dendrobium hercoglossum*, *Torenia fournieri*, *Dendrobium crumenatum* and *Grammatophyllum specinocum*.

The growth on PDA medium 7 days have 8.5-9 cm, the mycelia initially white-grey and then become black-brown. The conidia have no color with rods shape (Busarakham, 2001).

Previous study from India showed that the endophytic fungus, *Colletotrichum gloeosporioides* was isolated from *Justicia gendarussa*, a medicinal plant have ability to produce Taxol that is a potent anticancer drug used widely in the treatment of a variety of cancers (Gangadevi and Muthumary J., 2008).



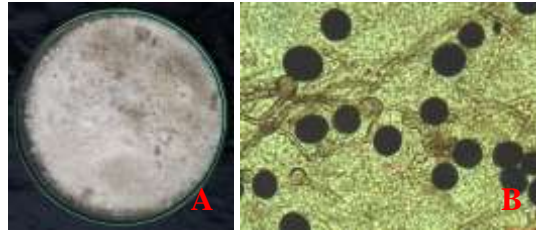
**Figure 4.** *Colletotrichum* sp. **A:** The colony culture on PDA 30 days and **B:** The spores under microscope 40X

#### 5. *Nigrospora* sp.

Habitat: *Dendrobium hercoglossum*

*Nigrospora* grows quit fast and produces woolly colonies on potato dextrose agar (PDA) at room temperature, on 7<sup>th</sup> days has 9 cm in diameter. Color of the colony is white initially and then becomes gray with black areas and turns to black eventually from both front and reverse. Spores (conidia) are produced singly on swollen urn-shaped conidiophores and are egg-shaped to flattened-spherical, black, and often have an equatorial colourless line or germ slit.

This species also can isolated from deep sea invironment at Arabian Sea and The secondary metabolites produced from this organism showed potent antimicrobial and anticancer activities with immediate application to cosmetics and pharmaceutical industries. (Arumugam *et al.*, 2015)



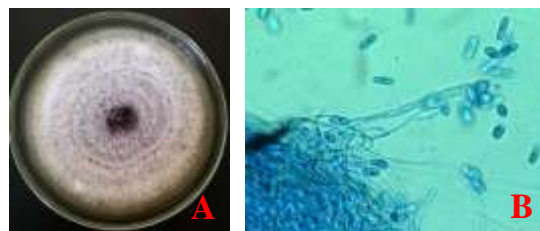
**Figure 5.** *Nigrospora* sp. **A:** The colony culture on PDA 30 days **B:** The hyphea and spores under microscope 40X

### 6. *Phoma* sp.

Habitats: *Cymbidium dayanum*, *Doritis pulcherrima* and *Grammatophyllum specinocum*.

The hyphae were growing rapidly, for 2 days that cultured on PDA medium have 6.5 cm in diameter. The hyphae first growth have white color and than become black-brown. Spores are colorless and unicellular. The pycnidia are black and depressed in the tissues of the host.

*Phoma* sp. are endophytic fungi that can isolated from many plants. Wang LW *et al.* (2012) isolated *phoma* sp. from chinese medicinal plant *Arisaema erubescens* and showed that the bioactive metabolites from this species can inhibited the growth of four plant pathogenic fungi (*Fusarium oxysporium*, *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, and *Magnaporthe oryzae*). This species also isolated from roots of cucumber in Korea and showed that have ability to produce Gibberellins and Indoleacetic Acid and Promotes Host-Plant Growth during Stress (Waqas M. *et al.*, 2012).



**Figure 6.** *Phoma* sp1. **A:** The colony culture on PDA 30 days and **B:** The spores and mycelium under microscope 40X

### 7. *Curvularia* sp.

Habitats: *Grammatophyllum scriptum* and *Doritis pulcherrima*.

The growth was quit fast for cultured on PDA medium for 2 days have 5.2cm in diameter. From the front, the color of the colony is white to pinkish gray initially and turns to black as the colony matures. From the reverse, it is dark brown to black. Conidia are pale brown, with three or more transverse septa (phragmoconidia) and are formed apically through a pore (poroconidia) in a sympodially elongating geniculate conidiophore similar to *Drechslera*. Conidia are cylindrical or slightly curved, with one of the central cells being larger and darker. Germination is bipolar and some species may have a prominent hilum (McGinnis, 1980).

Zakaria L. *et al.* (2010) isolated this species from *Oryza sativa*. Aharwal R.P. *et al.* (2014) also isolated this fungus from *Calotropis procera* (Linn.) R.Br. a widely used medicinal plant in India.



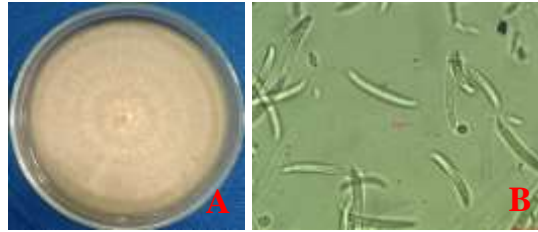
**Figure 7.** *Curvularia* sp. **A:** The colony culture on PDA 30 days and **B:** The spores under microscope 40X

#### 8. *Fusarium* sp.

Habitat: *Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobium palpebrae*, *Torenia fournieri* and *Dendrobium friedericksianum*.

The hyphae are white. The growth was quit fast, for cultured on PDA in room temperature, for 4 days have 4.9 cm in diameter. The conidia are fusiform, slightly curved, pointed at the tip. *Fusarium* is a filamentous fungus widely distributed on plants and in the soil. It is found in normal mycoflora of commodities, such as rice, bean, soybean, and other crops.

Previous study showed that *Fusarium redolens*, isolated from Himalayan yew can produce taxol, the anticancer drug (Garyali S, 2013). Sogra and Raj (2014) isolated *Fusarium oxysporum* from leaf of *Nothapodytes foetida*, ethyl acetate extract from this fungus show ability to inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*.



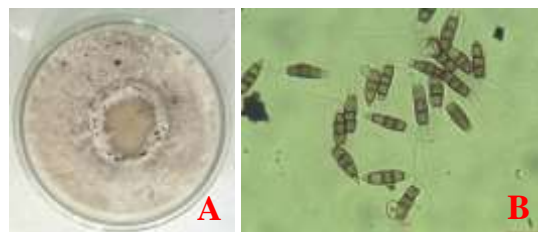
**Figure 8.** *Fusarium* sp. **A:** The colony culture on PDA 30 days and **B:** The spores under microscope 40X

### 9. *Pestalotiopsis* sp.

Habitat: *Dendrobium friedericksianum*, *Cymbidium dayanum* and *Grammatophyllum scriptum*.

The mycelia are white in color. This species have quit slow of growing. For growing on PDA in room temperature for 7 days have 5.5cm in diameter. Conidiophores (annellides) produced within compact fruiting structures (aecervuli or pycnidia). Spores (conidia) 4 to 5 celled, with the two or three central cells dark brown, and with two or more apical appendages or hairs.

*Pestalotiopsis* sp. is the common endophytic fungi that can isolate from orchids and other plants. They also isolated from other orchid varieties from other country such as Tempesta S. *et al.* (2011) isolated endophytic fungi from leaves of *C. mrcranthum* and *O. adendrobium* also found 29 strains of *Pestalotiopsis* sp. Wei J.G *et al.* (2007) isolated this species that associated with plants of Podocarpaceae, Theaceae and Taxaceae in southern China also obtained 24 species. Kumaran RS *et al.* (2010) isolated *Pestalotiopsis verisicolor* and *Pestalotiopsis neglecta* as endophytic fungi from Japanese Yew tree, *Taxus cuspidata* and indicated that they can produce taxol concentration induces increased cell death in cancer.



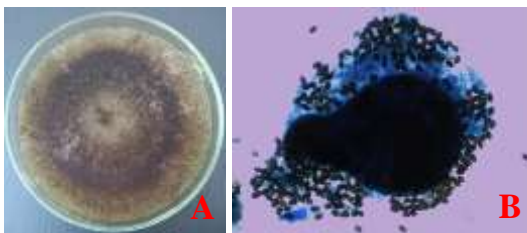
**Figure 9.** *Pestalotiopsis* sp. **A:** The colony culture on PDA 30 days **B:** The conidia under microscops 40X



### 10. *Corynascus* sp.

Habitat: *Cymbidium dayanum*

The fungus have quit fast in growth. Only 3 days the mycelia have 9.0 cm in diameter. It has black-white mycelia, produce lots of black spores and inside spores there are lots of little ascospores.

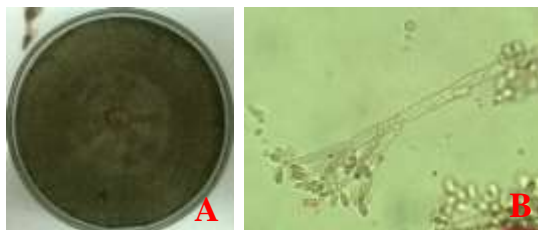


**Figure 10.** *Corynascus* sp. **A:** The colony culture on PDA 30 days and **B:** The spores and acospses under microscops 40X

### 11. *Cladosporium*-like fungi

Habitat: *Grammatophyllum scriptum*, *Cymbidium dayanum*, *Dendrobium palpebrae*, *Doritis pulcherrima* and *Dendrobium crumenatum*

The initial growth the mycelia have white color and then become black. The growth was quit fast. On 6<sup>th</sup> day of culture on PDA have 9 cm in diameter. The mycelia have branches and the lots of small spore.



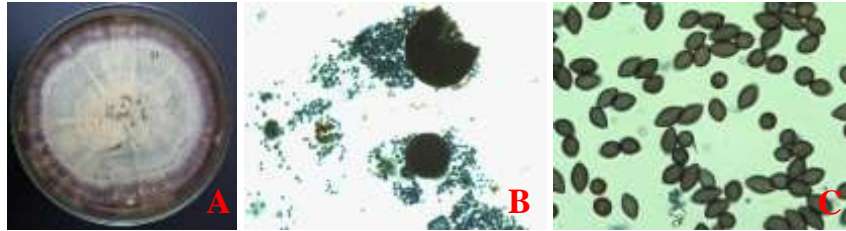
**Figure 11.** *Cladosporium*-like fungi **A:** The colony culture on PDA 30 days and **B:** The spores and mycelia under microscops 40X

### 12. *Achaetomium* sp.

Habitat: *Grammatophyllum scriptum*

The mycelia growth look like the yellow sheets and this fungus growth quit slow, cultured on PDA medium in room temperature for 7 days have 5.5 cm in

diameter. The spores are large with yellow in color. Inside the spores have many ascospores.



**Figure 12.** *Achaetomium* sp. **A:** The colony culture on PDA 30 days **B:** The spores under microscopes 10X and **C:** The ascospores under microscopes 40X

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