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# Molecular Identification of *Chaetomium* species from Soil in Vietnam

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**Abstract** This study reported some species of the *Chaetomiaceae* from collected soil samples in Vietnam. Soil samples were taken from dragon fruit crop cultivation in Tien Giang and Long An provinces in 2017. All species of *Chaetomiaceae* were isolated from soil samples by soil baiting technique. All species were identified based on the morphological characteristics and phylogenetic analysis of β-tubulin gene. At least four species of the *Chaetomiaceae* family were identified, including *Arcopilus aureus*, *Arcopilus cupreus*, *Chaetomium cochliodes* and *Chaetomium globosum*. These strains were strongly antagonistic activity against *Neoscytalidium dimidiatum* causing brown spot disease of *Hylocereus undatus* (white-fleshed pitahaya) in Vietnam. Further research would be done the control mechanism and acute and dermal toxicology test for environmental safety. Finally it maybe formulate as biological fungicide to control this serious disease in the field.

**Keywords:** *Chaetomium*, β-tubulin gene, dragon fruit, soil samples

## Introduction

In Vietnam, dragon fruit (*Hydrocereus undatus*) has been planted by the French over 100 years (Luders & McMahon, 2006). This variety is the most commonly cultivated in all regions of the country. In recent years, brown spot disease (white spot, yellow cladode disease) was considered the most serious disease for dragon fruit industry. In the field, the rate of incidence was 30 up to 70 percent (Nguyen *et al*., 2014). It was found that the causal agent of brown spot disease was *Neoscytalidium dimidiatum* (Nguyen *et al*., 2014; Vo *et al*., 2015). At present, the applications of beneficial microorganisms is a potential measure to control the plant diseases and improve for sustainable cultivation.

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Among the antagonistic species that can be used in the prevention of plant diseases, the *Chaetomium* spp. has great potential.

Approximately 7 species have been recorded in Vietnam. Le *et al*. (2005) have identified four species including *Chaetomium cupreum*, *Ch. globosum*, *Ch. mollicellum* and *Ch. cuminulorum* from cultivated soil of rice, maize, soybean and coffee. Thiep and Soytong (2015) have identified three species including *Ch. cochliodes*, *Ch. bostrychodes* and *Ch. gracile* from cultivated soil of tea, coffee and rubber. All these *Chaetomium* species were only identified based on morphological characteristics. There is not any nucleotide sequences submitted to the Genbank.

*Chaetomium* species are traditionally identified by morphological data, the type of terminal hair and lateral hairs or ascomatal hairs covering the ascomata, the shape and size of asci and ascospores according to von Arx *et al*. (1986) and Soytong and Quimio (1989). According to Wang *et al*. (2016b), 5' region of the β-tubulin gene (*tub2*) has been shown to be more effective than the internal transcribed space gene sequence for distinguishing *Chaetomium* spp. at the species level, so that in this study, T1/T2 primer pair (O'Donnell & Cigelnik, 1997) were used to multiply a 700-bp segment of the β-tubulin gene.

The objectives of this study was conducted to identify the species level of the *Chaetomiaceae* family from collected soil samples.

## Materials and methods

### *Research materials*

Soil samples were taken from dragon fruit crop cultivation in Tien Giang and Long An provinces in 2017. Soil samples were collected at a soil depth of 15-20 cm with 500 g/sample. Soil samples were contained in a plastic bags, labeled with survey informations and brought to the laboratory at the Microbiology Division, Agricultural Genetics Institute, Hanoi, Vietnam.

Water agar (WA), PDA (potato dextrose agar). Chemicals used in are derived from Vietnam, China, Japan and USA.

### *Isolation of Chaetomium spp.*

*Chaetomium* spp. were isolated by baiting technique according to the method described by Soytong (1991): the soil samples were dried at room temperature, finely grinded particles using porcelain pestles, then were placed to 90 mm diameter sterilized petri dishes, about 10 g of each soil sample per petri dish. The soil was moistened with sterile distilled water before baited with small pieces of sterilized filter paper (0.5 x 0.5 cm diameter). The soil plates

were incubated at room temperature condition. *Chaetomium* spp. on baits were daily observed and picked their ascomata to glass slide with small amount of sterilized water before spread on WA in a 90 mm diameter petri dish. The WA plates were incubated for 12 h at room temperature, then single colony was transferred onto PDA plates and isolated into pure culture.

***Molecular identification of Chaetomium spp.***

Total DNAs were extracted by CTAB (cetyltrimethyl ammonium bromide) method described by Doyle and Doyle (1987). Total DNAs were diluted in 50 µl TE buffer and stored at minus 20°C until use. T1/T2 primer pair (O'Donnell and Cigelnik, 1997) were used to multiply a 700 bp segment of the β-tubulin gene. The reaction was carried out in 25 µl volumes containing 1 µl genomic DNA, 0.5 µl dNTPs, 1 µl of each primer and 0.2 µl *Taq* DNA polymerase in 2.5 µl PCR buffer. PCR products were separated on 1.0% agarose gel in 1X TAE buffer.

PCR products were purified using the PureLink™ Quick Gel Extraction Kit (Invitrogen) according to the manufacturer's instructions. Based on the nucleotide sequences obtained, the database was searched for the Genbank using BLAST search online software at the National Center for Biotechnology Information (NCBI). Nucleotide sequences of the related species based on the β-tubulin gene were retrieved from GenBank (Table 1). *Achaetomium strumarium* (GenBank accession No. AY681238) was used as outgroup. All of the sequences were assembled using BioEdit, version 7.0.2 and aligned using ClustalX, version 1.83. Phylogenetic relationship was constructed by performing heuristic search under Neighbor-joining (NJ).

**Table 1.** *Chaetomium* species and isolates used in this study

| Type species | Isolate code | GenBank accession No. | Substrate/ Locality | Country |
|---|---|---|---|---|
| *Aropilus aureus* | CBS 153.52 | KX97446924 | Unkown | USA |
| *Aropilus aureus* | CBS 538.73 | KX976925 | Animal dung | East Africa |
| *Aropilus cupreus* | CBS 560.80 | KX976926 | Animal dung | Canada |
| *Aropilus fusiformis* | CBS 484.85 | KX976927 | Animal dung | USA |
| *Aropilus fusiformis* | CBS 485.85 | KX976928 | Wood chip | Canada |
| *Chaetomium afropilosum* | CBS 145.38 | KT214751 | Unkown | Unkown |
| *Chaetomium ascotrichoides* | CBS 113.83 | KC109770 | *Gossypium humitectum* | Argentina |
| *Chaetomium* | CBS 110.83 | KC109771 | Soil | Israel |

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|---|---|---|---|---|
| *ascotrichoides* | | | | |
| *Chaetomium citrinum* | CBS 693.82 | KT214764 | Soil | Japan |
| *Chaetomium cochliodes* | CBS 155.52 | KC109772 | Animal dung | USA |
| *Chaetomium cochliodes* | CGMCC 3.9440 | JN256145 | Tuber of *Panax notogingseng* | China |
| *Chaetomium cochliodes* | DTO 013-C2 | KX976947 | Unkown | Holland |
| *Chaetomium cochliodes* | DTO 089-E2 | KX976948 | Unkown | Holland |
| *Chaetomium elatum* | CBS 910.70 | KC109775 | Leaves and dead stem of *Ammophila arenaris* | Germany |
| *Chaetomium elatum* | CBS 374.66 | KC109776 | Decomposing leaf | USA |
| *Chaetomium fimeti* | CBS 139034 | KT214736 | Soil | Germany |
| *Chaetomium interruptum* | CBS 126660 | KT214741 | *Triticum aestivum* | Iran |
| *Chaetomium globosporum* | CBS 108.83 | KC109768 | *Triticum aestivum* | India |
| *Chaetomium globosum* | CBS 164.62 | JN256190 | Unkown | Poland |
| *Chaetomium globosum* | CBS 371.66 | JN256148 | Paper | USA |
| *Chaetomium globosum* | CBS 147.60 | JN256179 | Raincoat | USA |
| *Chaetomium graminiforme* | CBS 506.84 | KT214761 | *Acer* sp. | USA |
| *Chaetomium pseudocochliodes* | CGMCC 3.9469 | JN256194 | Rhizosphere of *Panax notogingseng* | China |
| *Chaetomium. madrasense* | CBS 315.74 | KC109769 | Rhizosphere of *Pennisetum typhoides* | India |
| *Chaetomium nozdrenkoae* | CBS 163.62 | KT214733 | Soil | Russia |
| *Chaetomium nozdrenkoae* | CBS 809.68 | KT214734 | Soil | Germany |
| *Chaetomium tenue* | CBS 139.38 | KT214745 | Unkown | Unkown |
| *Chaetomium tenue* | CBS 138.38 | KT214746 | Unkown | Unkown |
| *Chaetomium telluricola* | CBS 151.59 | KT214759 | Soil | England |
| *Chaetomium umbonatum* | CBS 293.83 | KT214752 | Soil | Canada |
| *Achaetomium strumarium* | CBS 333.67 | AY681238 | Soil | China |

## Results

### *Morphological identification of Chaetomium spp.*

Identification of *Chaetomium* species are usually considered morphological characters as the type of terminal hair and lateral hairs or ascomatal hairs covering the ascomata, the shape and size of asci and ascospores.

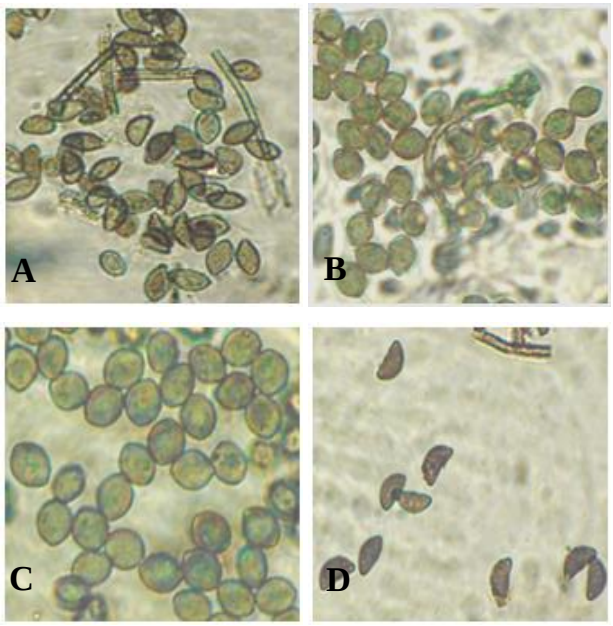


**Figure 1.** Ascospore of *Chaetomium* spp. isolated from soil samples on PDA
A) Isolate TG-CG.07; B) Isolate TL-LA.01; C) Isolate TG-CG.07; D) Isolate TG-CG.02

The result showed that the isolates collected from soil in 2017 were morphological identified into 4 species as follows *Chaetomium aureum (Syn. Arcopilus aureus*), *Ch. cupreum (Syn. Arcopilus cupreus*), *Ch. cochliodes* and *Ch. globosum*.

### *Molecular identification of Chaetomium spp.*

At present, for identification of *Chaetomium* spp. based only on morphology is not sufficient and even an analysis of the internal transcribed space gene. The molecular phylogeny of effective isolates of *Chaetomium* spp. was confirmed by molecular phylogenetic to species level, in this study, T1/T2 primer pair (O'Donnell and Cigelnik, 1997) were used.

The PCR results showed that the DNA band fragments appeared on the agarose gel were clear, not smear, with a size approximately of 700 bp (data not shown). These PCR prducts were further sequenced in the *tub2* gene for the identification of the species level.

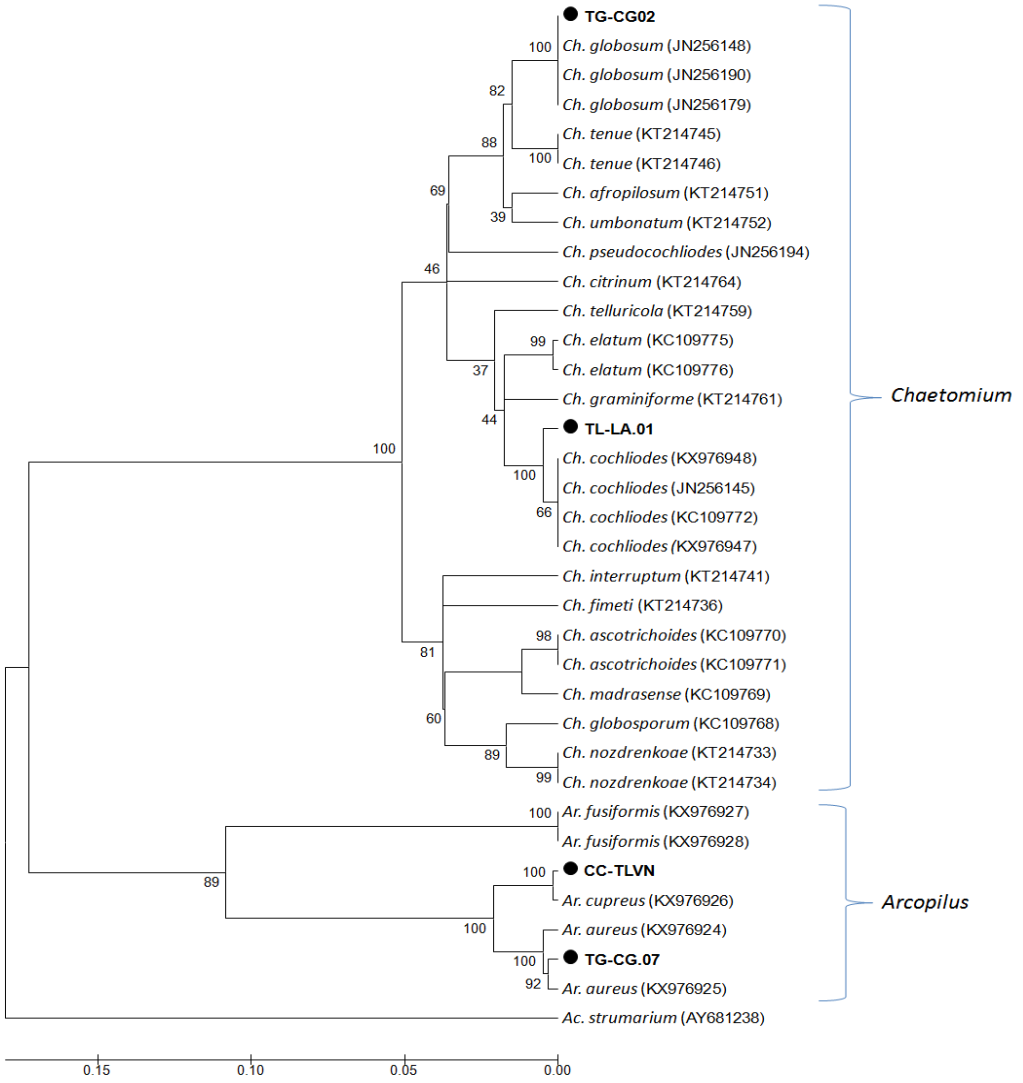


**Figure 1.** Phylogenetic tree was contructed by Neighbor-joining method based on the 5' region of the *tub2* gene of the type species. Samples isolated from soil in Vietnam (black dots). The bar represents the genetic distance. Value in nodes is the bootstrap statistics value as % (1.000 iterations).

The results of phylogenetic analysis showed that the four fungal species in this study distinguished into distinct clusters of species level. Two species (*Ch. cochliodes* and *Ch. globosum*) belonging to *Chaetomium* genus and two species (*Ar. aureus* and *Ar. cupreus*) belonging to *Arcopilus* genus, *Chaetomiaceae* family. Based on morphological characteristics and molecular analysis,

## Discussion

Chaetomium isolates were morphologically identified according **to** (Arx *et al*., 1986; Soytong and Quimio, 1989). Wang *et al*. (2016a) ranked five species *including Ch. aureum*, *Ch. cupreum*, *Ch. fusiforme*, *Ch. flavigenum* and *Ch. turgidopilosum* belonging to the *Chaetomium* as a new genus, *Arcopilus*, the *Chaetomiaceae* family, and the five species are renamed *Ar. aureus*, *Ar. cupreus*, *Ar. fusiformis*, *Ar. flavigenus* and *Ch. turgidopilosus*. According to Wang *et al*. (2016b), 5' region of the β-tubulin gene has been shown to be more effective than the internal transcribed space gene sequence. Thus, this study showed that the nucleotide sequences of the *tub2* gene were useful to taxon species level of *Chaetomium* spp., as previously reported (Wang *et al*., 2016a, 2016b).

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