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# Morphotypes of Mycorrhizal Fungi of Cymbidium Species

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## ABSTRACT

*Mycorrhizal association is known to be important to orchid species and a complete understanding of the fungi that form mycorrhizas is required for orchid ecology and conservation. Cymbidium is a orchid found in Eastern and Western Ghats of India at high altitudes. Previously, we found the genetic diversity of this species has been reduced recent years due to habitat destruction and fragmentation, but little was known about the relationship between this orchid species and the mycorrhizal fungi. The Rhizoctonia-like fungi are the commonly accepted mycorrhizal fungi associated with orchids. In this study, morphotypes of the Rhizoctonia-like fungi associated with Cymbidium species were investigated and their isolates were obtained.*

***Key words:*** *endophytes, Cymbidium species, Morphotypes and Rhizoctonia-like fungi.*

## INTRODUCTION

The mycorrhizal association is ubiquitous but very important symbiosis in nature, which plays an essential role in the maintenance of most terrestrial ecosystems[1]. Over 90% of all plant species can form mycorrhizas with different kinds of fungi and the existence of mycorrhizal fungi can confer to their hosts many adaptive advantages via improved water and nutrient or minerals uptake from the soil[2,3,4], enhanced plant growth[5,6] , reduced toxic element accumulation[1,7] and increased resistance to pathogen damage[8]. The Orchidaceae, which is one of the largest and most diverse plant families, is distributed worldwide[9]. However, many orchid species have suffered dramatic declines in distribution and some species have become rare and endangered in recent decades[10]. Mycorrhizal association is known to be important to orchids because they depend on the presence of suitable fungal partners for seed germination and seedling development[11,12]. Therefore, a complete understanding of the mycorrhizal fungi of many threatened orchid species is required for conservation action plans[13].

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The study of the earliest diverging orchid lineages and distribution of mycorrhizal fungal associates across orchid phylogeny supported that the ancestral state is an association to the Rhizoctonia-like fungi lineages[14] and orchid mycorrhizas are predominantly represented by associations between photosynthetic plants and Rhizoctonia-like fungi[13]. The Rhizoctonia-like fungi includes members of the Ceratobasidiaceae, Sebacinales and Tulasnellaceae[13,14]. Many fungi that have been isolated from orchid roots have been identified as Rhizoctonia-like. Members of that group do not form asexual spores and all share certain distinctive vegetative characters. Six Epulorhiza species have been described based on the shape and the dimensions of monilioid cells[15].

Cymbidium genus is distributed in tropical and subtropical Asia (such as northern India, China, Japan, Malaysia, the Philippines, and Borneo) and northern Australia. The larger flowered species from which the large flowered hybrids are derived grow at high altitudes[16]. Several species of Cymbidium occurring in Eastern North Asia, e.g., Cymbidium goeringii and C. kanran, are important in oriental floricultural industries. However, these could not properly performed without any OM fungi. Recently, the inoculation of OM fungal isolates to Cymbidium hybrids was reported to increase survival rates, and they protected plants from root rots or soil pathogens[17]. The present work involves isolation and identification of mycorrhiza of *Vanda* and further studies can be done by molecular methods.

## MATERIALS AND METHODS

### Study area ad Location

Roots were procured from three different regions of naturally growing plants of Cymbidium that is shown in the table.1.These were collected in October to January during their active vegetative growth, stored in the paper bags/ziplock and transferred to the laboratory.

**Table 1. Location of the different populations of *Cymbidium* species**

| Population Code | Location |
|---|---|
| PU14 | Kodai |
| KEKU | Kulitholu, Kerala |
| THO | Thodu hills, Kerala |

### Isolation of fungal endophytes

Potential mycorrhizal fungi were isolated from the orchid plants and identified from pure culture of *Cymbidium species*, unlike many other temperate, terrestrial orchids which have thick roots and often produce abundant pelotons, has few active pelotons suitable for isolation. Thus, endophytic fungi were isolated from single hyphal tips emerging from sterilized root portions as in the isolation method described[18]. Three roots per plant were carefully cleaned from the soil under running water, surface-sterilized in 0.1% (v/v) Mercuric chloride and 75% (v/v) ethanol for 3 min and 5 s respectively, and subsequently washed three times in sterile distilled water. Root sections of 3–5 mm thickness were obtained by cutting and placed in a petridish with potato dextrose agar (PDA). Petri dishes were incubated at 25°C in the dark and observed for fungi growing every 2 days for at least 3 weeks. The growing colonies were separated onto fresh media for purity and this process was repeated three times.

## RESULTS AND DISCUSSION

### Morphological identification

Classification of the endophytic fungi was based on their growth rate and morphological characteristics, including colonial morphology, production of conidiogenous cells, conidial size and dimension on PDA medium[19] and similar isolates were grouped into one morphotype. The Rhizoctonia-like fungal endophytes were recognized by the following characteristics: hyphas hyaline with constricted branch points, 2.5–9 cm diameter; submerged growth in PDA; ellipsoid, globose or irregular monilioid cells; colony creamy white to pale tan or orange, rubbery or leathery in appearance and texture[20,21,22] .

**Table 2. Morphotypes of fungal isolates from the *Cymbidium* species**

| Morphotype | Colony color | Colony texture | Growth rate (cm/day) | Aerial mycelium | Conidial shape | Conidia size |
|---|---|---|---|---|---|---|
| I | Creamy white | Bushy | 3.25 | Absent | Circular | - |
| II | Brown to white | Leathery | 6.5 | Absent | Irregular, cylindrical | - |
| III | Greenish brown to white | Brushy, Slightly loose | 6.2 | Absent | Elliptic irregular | - |

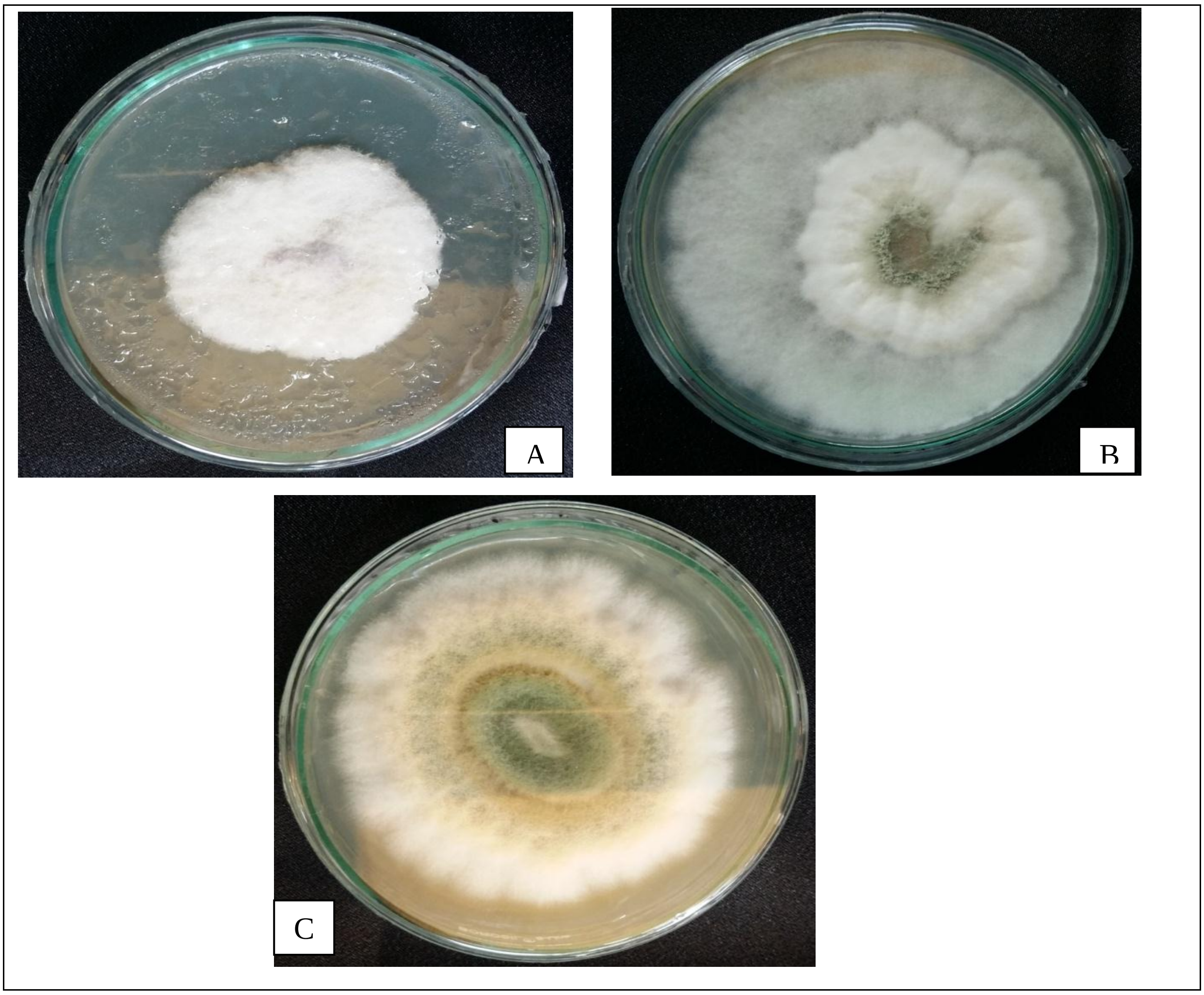


**Fig. 1: The morphotypes of endophytes isolated from *Cymbidium* species**

After isolation and purification, isolates of endophytic fungi from the plants were obtained. According to their morphological characters and growth rate on PDA medium, fungal isolates were classified into different morphotypes (Table 2). Morphological characters and detailed descriptions of the morphotypes were given in Fig. 1 and Table 2. Only the Rhizoctonia-like isolates were taken and subjected to further studies for DNA extraction and phylogenetic analysis.

## CONCLUSION

Mycorrhizal association is known to be important to orchid species and a complete understanding of the fungi that form mycorrhizas is required for orchid ecology and conservation. Thus, the morphotypes of the Rhizoctonia-like fungi associated with *Cymbidium* species were obtained and further conformations can be done through molecular methods.

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