

Morphotypes of Mycorrhizal Fungi of Vanda 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. Vanda is a widespread orchid in Eastern and Western Ghats of India. 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 Vanda species were investigated. Among the endophytic fungal isolates obtained, Rhizoctonia-like fungi were identified based on morphological characters and further conformations can be done through molecular methods.

Key Words: endophytes, *Vanda 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]. 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, Sebaciales and Tulasnellaceae [13,14].

Vanda commonly known as 'blue vanda' is a floriculturally important and endangered species of the epiphytic and monopodial orchids. It is found at elevations of 1000–1500 m and is endemic to the state of Meghalaya and Manipur in India and northern range of Thailand and Burma. Flowers are known to have a long shelf life (2–3 months). This beautiful orchid has been extensively used to progenerate a vast variety of remarkable hybrids [15]. The species is also known for its ethnobotanical importance; its flowers are used as pectoral, and the juice from its leaves is used to cure diarrhoea, dysentery and dermal disorders. It has been listed as an endangered species of Red Data Book on Indian Orchidaceae-1 and as threatened by the International Union for Conservation of Nature and Natural Resource. As a result, the Committee of International Trade in Endangered species of World Fauna and Flora has imposed a ban on its trade [16].

As only limited information is available for mycorrhizal fungi in epiphytic orchids, which constitute a majority of orchids, it is important to isolate and identify orchid mycorrhiza and understand orchid-fungus relationship in epiphytic orchids. Keeping this in view, the present studies were planned with an objective to re establish the in

in vitro symbiotically raised seedlings back to their natural habitat so that *Vanda* can be restored to protected habitats. Fungus from *Vanda* has been isolated and morphologically identified [17]. However, molecular work is still lacking and for accurate identification, these techniques are considered more authentic [18]. 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 and Location

Roots were procured from four different regions of naturally growing plants of *Vanda* 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 *Vanda species*.

Population Code	Location
PU14	Kodai
KMO	Kolli hills
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 *Vanda 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 by [19]. 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

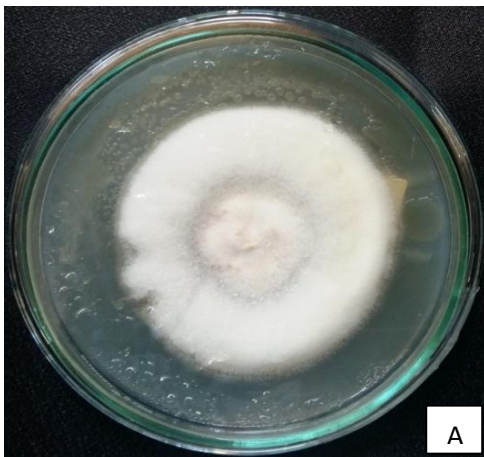
Morphological identification

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 [20] 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 μ m 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 [21–23].

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

Morphotype	Colony color	Colony texture	Growth rate (cm day ⁻¹)	Aerial mycelium	Conidial shape	Conidia Size
I	creamy white	leathery, compact	6.25	absent	circular	–
II	brown to white	bushy	5.5	absent	elliptic cylindrical	–
III	white	bushy, slightly loose	5.2	absent	irregular	–
IV	white	loose	4.9	absent	ovate, cylindrical	–
V	white	cottony, slightly loose	5.2	absent	elliptic	–
VI	light brown to white	cottony, stepwise	8.7	absent	clavate, elliptic	–
VII	brown	felted, compact	4.37	absent	irregular	–



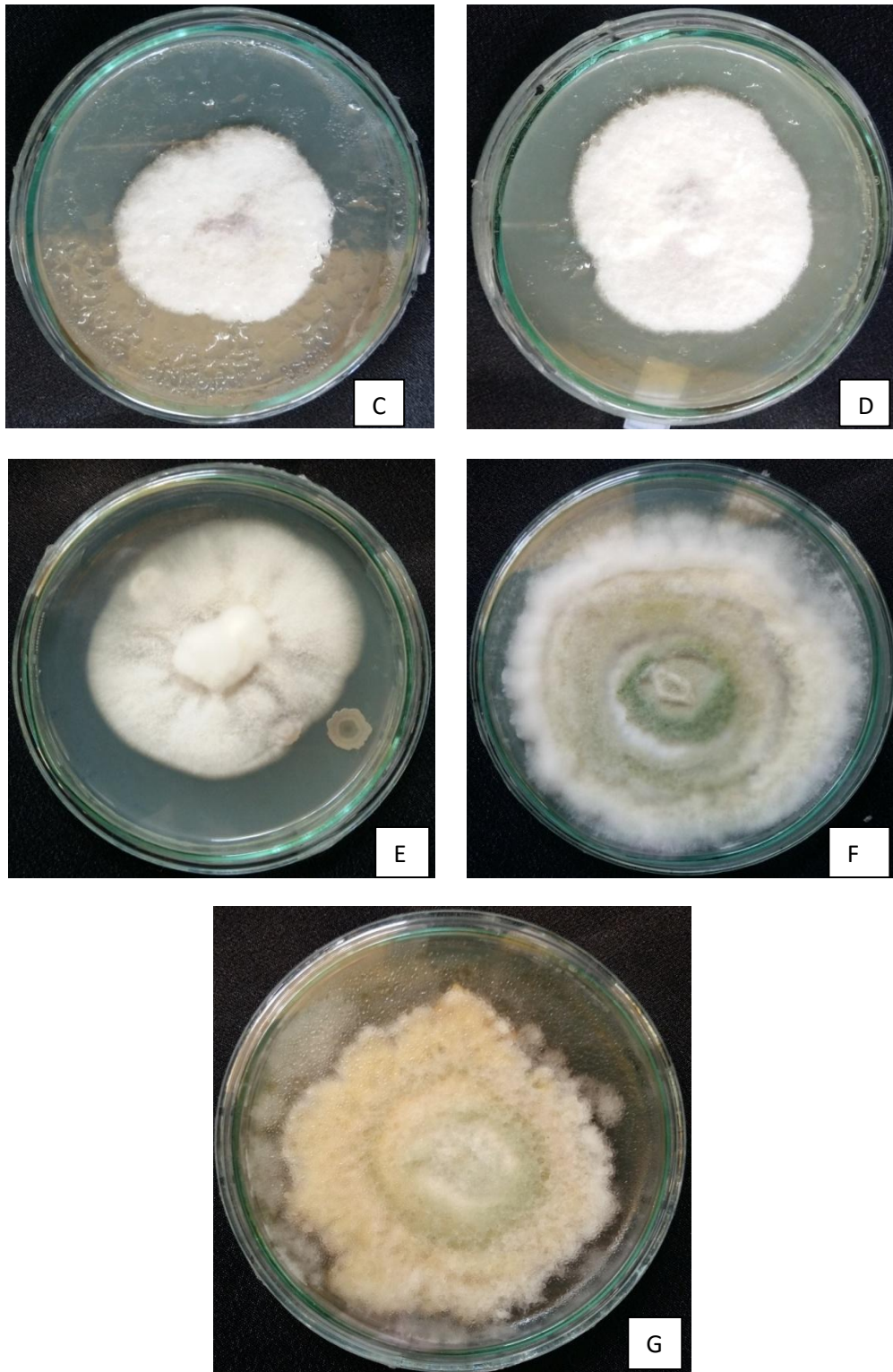


Figure 1. The morphotypes of endophytes isolated from *Vanda species*.

Fungal distribution and morphological diversity

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 *Vanda* species were obtained and further conformations can be done through molecular methods.

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