Asian J Biol Life Sci., 2025; 14(2):1-5.
https://ajbls.com

Research Article

Exploring Mycorrhizal Associations in Orchids: Isolation, Identification, and Morphological Insights

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ABSTRACT

Background/Purpose: Epiphytic orchids, such as *Aerides odorata* L., rely on symbiotic mycorrhizal fungi for nutrient acquisition, germination, and early growth due to the nutrient-deficient nature of their seeds. The hypothesis of this study was that Rhizoctonia spp. are the primary mycorrhizal fungi associated with A. odorata and that their growth characteristics vary depending on the culture medium. **Materials and Methods:** To test this, fungal samples were isolated from *A. odorata* and cultured on four different media: Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Malt Extract Agar (MEA), and Czapek Dox Agar (CZA). **Results:** Microscopic examination confirmed the presence of Rhizoctonia spp., characterized by septate hyphae and the absence of conidia, indicating asexual reproduction. Fungal growth was evaluated, with PDA and SDA showing the most rapid and extensive growth, while CZA exhibited the slowest and most restricted growth. **Conclusion:** The results suggest that Rhizoctonia spp. play a significant role in the symbiosis of *A. odorata*, and that media selection is crucial for optimizing fungal cultivation. These findings contribute to improving ex situ fungal culture techniques, which are vital for the conservation and sustainable propagation of *A. odorata* L.

Keywords: *Rhizoctonia* spp., Mycorrhizal Fungi, *Aerides odorata* L., Culture media, Fungal proliferation.

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Received: 07-03-2025;
Revised: 18-04-2025;
Accepted: 23-06-2025.

INTRODUCTION

The Orchidaceae, an orchid family, is one of the largest and most diverse groups of flowering plants globally, with over 35,000 species and 800 genera; India alone has 1141 species and 166 genera.[1] Orchids have a fascinating connection with mycorrhizal fungi, which are essential for their seed production. This dependence introduces major difficulties for their growth, both in cultivated settings and in nature. Unlike the seeds of most plants, orchid seeds lack conventional endospermic reserves. Instead, they depend on these helpful fungi for nutrient intake and protocorm growth.[2] However, identifying and cultivating these fungi remains a major hurdle to conservation efforts and the extensive distribution of orchids. Obstacle for isolation and cultivating these fungi that holds up large scale propagation of orchids and conservation of these species is prohibitive. A void point is currently a lack of a study on appropriate culture media for efficient growing orchid related mycorrhizal fungi in order to optimize fungal assisted germination.

Establishment of ideal culture condition is necessary to isolate and cultivate orchid associated mycorrhizal fungi. The media plays a very important role on the development and activity of these fungi. Nutrients, pH levels, and the availability of carbon and nitrogen are important elements that affect the fungi's growth and symbiotic effectiveness.[3,4] Spectacularly, media rich in ingredients of high sugar and poor in nutrients, such as Sabouraud Dextrose Agar can induce fungal growth. However, nutrient dense substrates that can promote sporulation and enzyme production are Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA).[5,6] *Aerides odorata* L. (*A. odorata* L.), one epiphytic orchid native to India is sought after for its horticultural, as well as its medicinal properties, were the subject of one such investigation. Nevertheless, *A. odorata* L. is an important part of the environment and is at risk due to destruction of the habitat and overharvesting so is in need of preservation.[7] To help in optimizing the fungus isolation and culture conditions for mycorrhizal fungi, this study was conducted.

MATERIALS AND METHODS

Chemicals and Media

All of the analytical grade substances used in the investigation were provided by HiMedia Laboratories Pvt. Ltd., Fungal isolation and growth optimization were carried out using Czapek Dox Broth, Sabouraud Dextrose Agar (SDA), Malt Extract

OPEN ACCESS

DOI: 10.5530/ajbls.20251487

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Powder and Potato Dextrose Agar (PDA) as culture media. Four standard medium types that permit fungal development the best were made. The media were checked and autoclaved for 15 min before being used at 121°C.[8]

Plant Materials

Specimens of *Aerides odorata* L. were collected from Panjab University, Orchid House, Chandigarh, India, adhering to stringent handling protocols to maintain the integrity of the specimens during the collection process.

Fungal isolation

In a zip lock bag we collected the roots and transport it to Botany Laboratory of Chandigarh University. Orchid samples were washed under running tap water for 5-10 min. and 3-5 rinses with sterile distilled water to get rid of the dust, the debris and to eliminate any residual chemicals and make the samples sterile.[9,10] Four different culture media were prepared to promote fungal isolation (Potato Dextrose Agar (PDA), Czapek Medium, Sabouraud Dextrose Agar (SDA) and Malt Medium). Sterile all media were poured into sterile petri dishes, allowed to solidify, and all media were sterilized prior to use. Specimens of *A. odorata* L. was then transferred to a laminar airflow chamber. In a similar process, a sterilized needle and blade were used to cut (or excise) a very thin section of the specimen, which was then placed on the solidified medium in each of the Petri dishes. Fungal growth was encouraged by incubating the inoculated samples at 25°C.[11,12]

Subculturing of Fungal Isolates

The fungal colonies were analyzed for its morphological characteristics and growth patterns to determine after 5-6 days incubation period. The actively growing fungus mycelia were transferred to new plates[13] containing four different culture media: Potato Dextrose Agar (PDA), Czapek Media, Sabouraud Dextrose Agar (SDA) and Malt Extract Media. Under aseptic conditions using a laminar airflow chamber, a piece of the actively growing fungal colonies was carefully transferred with a sterilized inoculation needle to fresh plates of PDA, Czapek Medium, SDA, and Malt Medium. After this, the subcultured plates were incubated at 25°C for 5-7 days at which point there is further fungal growth so that we can be thorough in our examination. This method ensured that the prevailing fungal strains could be isolated from the total population and, at the same time, were in ideal environment with which to continue their development in various conditions that were rich in nutrients.

Identification of orchid mycorrhizal fungi

After the production of fungal colonies, aseptically small portions of live mycelial growth were transferred to a clean glass slide with a sterilized needle. Each sample was then carefully placed on top of a coverslip in order to make optimal observation possible. The morphology of the fungi was analysed under a compound microscope at 40X magnification; fungal morphology included hyphal structure; spore formation; and reproductive structures. Morphological characteristics of the fungal isolates were used for their identification according to standard taxonomic keys.[6-14]

RESULTS AND DISCUSSION

Fungal Morphology and Identification

Fungal morphology was determined by microscopic analysis of the fungal isolates which included thin thread like septate hyphae having distinct branching patterns. Nodular structures are present, the formation of sclerotia is a known survival strategy of *Rhizoctonia species*. It is important to note, however, that the absence of spore or conidia forming structures confirmed the asexual reproductive character of *Rhizoctonia*. Most green orchids are associated with rhizoctonia fungi, which belong to a polyphyletic basidiomycetous group that includes the Tulasnellaceae, Ceratobasidiaceae (Cantherellales) and Serendipitaceae families.[15] Most of these fungi fall under the category of basidiomycetes in the Rhizoctonia complex.[16-19] The results from this study are consistent with previous studies of the low prevalence of *Rhizoctonia spp*. in mutualisms with fungi in orchids and described in more details *Rhizoctonia* was an important role to facilitate the exchange of nutrients between the fungal and orchid partner.[20] Without the mycorrhizal fungi partner, germination of orchid seeds is dependent on this mutualistic interaction and the orchid seeds have limited nutrient content.[2,20]

Comparative Growth Analysis on Different Culture Media

To determine the optimum medium for fungal growth, Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Malt

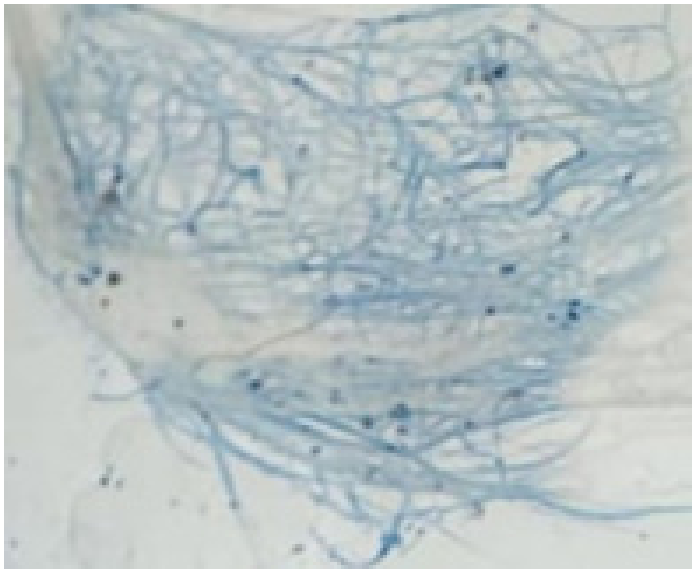

Figure 1: Roots of *Aerides odorata* L. have mycorrhizal fungi with fungal hyphae and spores were observed.

Extract Agar (MEA) and Czapek Agar (CZA) were used to culture the fungal isolates. The morphological features, growth patterns, growth extent and colony texture varied with different media (Table 1; Figure 1).

On MEA. By day 2, the first white colonies had formed, expanding to cottony mass of colonies by day 5. The gradual increase in the size and density of colonies indicates a favourable condition for fungal growth in MEA, although with a relatively lower rate than PDA and SDA.

On SDA, fungal growth was vigorous, with small colonies appearing on day. After 4 days the colonies were fluffy cottony structure and spread across large portion of the Petri dish. After six days, the colonies expanded significantly, indicating that SDA is well-suited for the growth of fungi. This supports previous studies,[6] stating that SDA's high carbohydrate content favours strong fungal proliferation.

Growth on Czapek Agar (CZA) was significantly slower and less dense compared to other media. Initial colony development was delayed until the third day, and growth remained limited throughout the duration of the study. The reduced fungal proliferation observed on CZA suggests that its nutrient composition may not be ideal for *Rhizoctonia* spp. These results align with the findings,[21] who noted that CZA is selective and favors slow-growing fungi (Table 2, Figure 2).

Potato Dextrose Agar (PDA) showed fastest and largest fungal growth was promoted by PDA. Colony formation first appeared on second day and fifth day were large cottony colonies filling much of the plate. The reason for PDA's high effectiveness as a growth medium was most likely its high carbohydrate content. This concurs with previous research which determined PDA as one of the best media for fungal culture.[5] Growth pattern a similar kind reported by previous authors.[16]

Comparative Summary of Fungal Growth on Different Media

The results showed that PDA and SDA are the most productive media for fungal growth, MEA promotes moderate development, whereas CZA is slowest for fungal proliferation. According to earlier studies on fungal culture media, these results are consistent, indicating the importance of one's choosing an appropriate medium, based on experimental purposes.[6]

Implications for Orchid Conservation and Future Research

The result of this present research indicates the major role played by *Rhizoctonia spp*. in the symbiotic relationship with *Aerides odorata* and the necessity of refinement in fungal culture techniques for maintaining the conservation of orchids. *Rhizoctonia's* capacity to invade orchid roots suggests its potential for use in ex situ propagation. Singh and Duggal emphasize the use of mycorrhizal-assisted orchid cultivation as a conservation strategy that may have been missing from this research, but it serves to support it.[22] In addition, future studies should focus to optimize culture conditions;[23] such as the use of growth stimulants or co-culturing with beneficial bacterial species.

Table 1: Composition of Different Culture Media Used in the Study.

Medium	Components	Quantity (per 100 mL distilled water)
Potato Infusion		
Potato Dextrose Agar (PDA)	Dextrose	3.9 g
Agar		
Sucrose		
Sodium Nitrate		
	Magnesium Sulfate	3.5 g
Czapek Medium	Potassium Chloride	
Ferrous Sulfate Dipotassium Phosphate		
	Agar	2 g
Dextrose		
Sabouraud Dextrose Agar (SDA)	Peptone	6.5 g
Agar		
Dextrose		
Malt Medium	Peptone	5.0 g
	Agar	2 g

Table 2: Summary of growth pattern.

	Extent of Growth	Growth Pattern	Type of Growth	Color
PDA (Potato Dextrose Agar)	+++++	Well-defined circular colonies	Extensive and dense	Milky white
Czapek Media	++	Small colonies	Slow and limited	White
MEA (Malt Extract Agar)	+++	Smooth texture	Moderate	White
SDA (Sabouraud Dextrose Agar)	++++	Uniform spread	Moderate to extensive	White to cream

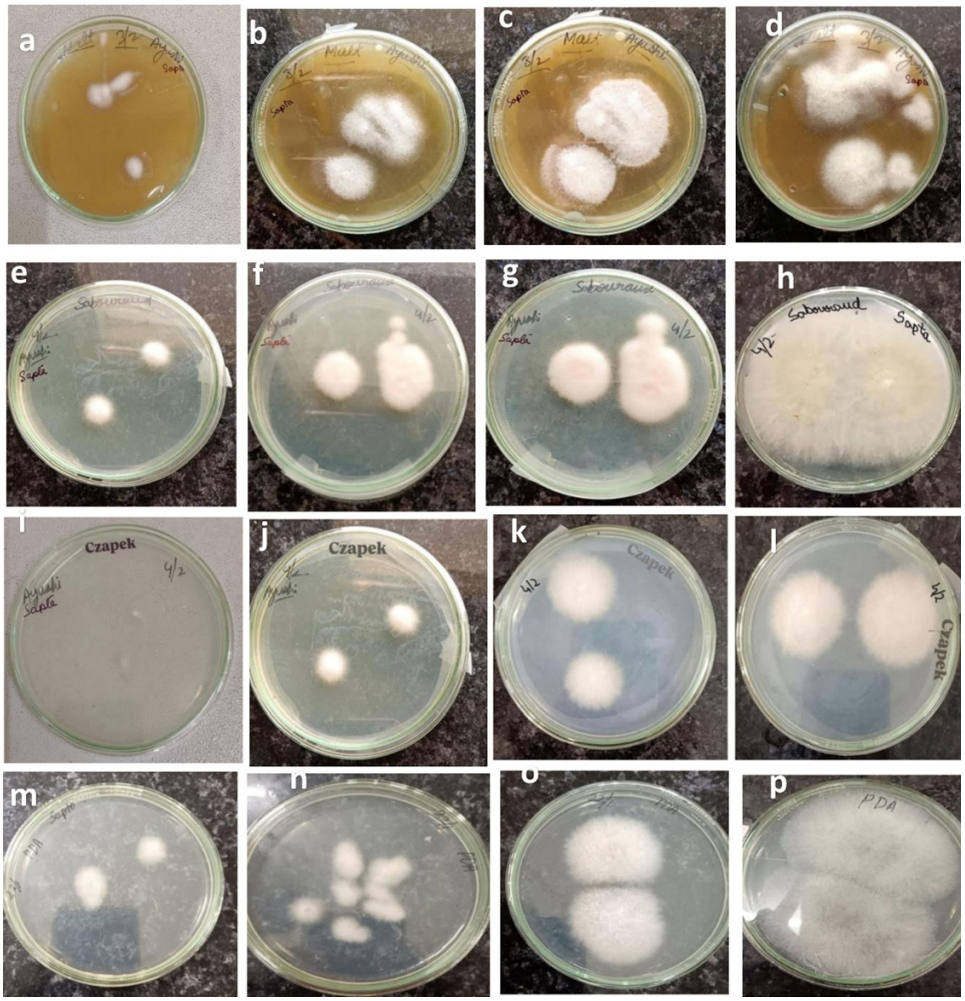

Figure 2: (a-d): Malt Medium (a fungal growth at 48 HAI (hours after inoculation (b) 72 HAI (c) Mature fungal growth 96 HAI (4th day) (d) 120 HAI (day 5) and a well-structured mycelium widely spread in the media. (e-h): On Sabouraud Dextrose Agar. E 48 HAI (Day 2) (f) 96 HAI (Day 4) (g) 120 HAI (Day 5) h) 144 HAI (Day 6). (i-l) On Czapek Dox Agar (CZA) (i) 48 HAI (Day 2) (j) 72 HAI (Day 3 (k) 96 HAI (Day 4) (l) 120 HAI (Day 5) (m-p): On Potato Dextrose Agar (PDA); (m) 24 HAI (Day 2); (n)72 HAI (By Day 3) (o) 96HAI (On Day 4), (p) 120 HAI (Day 5) and PDA shows rapid and dense growth.

CONCLUSION

This study concludes by highlighting the importance of compatibility variables, specifically the symbiotic interaction with mycorrhizal fungi, in orchid growth and development. For effective cultivation, the results highlight the significance of appropriate fungal relationships, ambient factors, and substrate selection. Enhancing propagation methods and supporting orchid conservation initiatives can be achieved by more research on optimising these compatibility factors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The research was conducted within our available resources and without specific financial support from any funding agency.

ABBREVAITIONS

ABM: *Aerides odorata* L.; CZA: Czapek Agar; HAI: Hours After Inoculation; HoD: Head of Department; MEA: Malt Extract Agar; PDA: Potato Dextrose Agar; SDA: Sabouraud Dextrose Agar; spp.: Species (plural); L.: Linnaeus; Pvt. Ltd.,: Private Limited; min: Minutes; °C: Degree Celsius; mL: Milliliter; g: Gram; vs: Versus; ***ex situ***: Off-site (outside the natural environment).

AUTHOR'S CONTRIBUTION

Both the authors have contributed to the research work and Research paper.

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Cite this article: Bhattacharjee S, Attri LK. Exploring Mycorrhizal Associations in Orchids: Isolation, Identification, and Morphological Insights. Asian J Biol Life Sci. 2025;14(2):x-x.