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Morphological and Anatomical Identification of *Rhizoctonia* Mycorrhizal from Five *Dendrobium* Sp.

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Abstract
Orchids are one of the plants that do not have food reserves (endosperm), so they require mycorrhizal fungi (Orchid mycorrhizal fungi / OMF) to help absorb nutrients and support seed germination. A more detailed understanding of the mycorrhizal character of each orchid species is very necessary because each orchid has a different OMF to support more optimal cultivation. This study aims to determine and identify the types of OMF morphologically and anatomically belonging to the *Rhizoctonia* mycorrhizal group that are able to associate with five species of *Dendrobium* sp. orchids, namely *Dendrobium spectabile*, *Dendrobium lineale*, *Dendrobium aphyllum*, *Dendrobium discolor*, and *Dendrobium fimbriatum*. The method used is the observation method by taking 150 OMF samples. Based on the results of cell nucleus observations, isolates from *D. aphyllum* have the highest percentage of binucleate cells (80%), so they are considered the most stable and have strong potential as functional mycorrhizae. In contrast, *D. discolor* had the lowest binucleate value (44%), indicating a weaker symbiotic ability compared to other isolates. Overall, this study demonstrates that host orchid species influence the characteristics of *Rhizoctonia* mycorrhizal. This information can be used as a basis for developing orchid cultivation technology and further research through molecular analysis.

Keywords: anatomy; *Dendrobium* sp.; morphology; *Rhizoctonia* mycorrhizal

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Introduction

Orchids are a highly sought-after ornamental plant due to their high economic value. *Dendrobium* is the largest genus in the orchid family, encompassing nearly 900 species and approximately 30,000 to 35,000 species worldwide, excluding Antarctica. In Indonesia, the *Dendrobium* family dominates, with approximately 6,000 orchid species (1). Orchid growth and development are highly dependent on the presence of mycorrhizal fungi, which live in symbiosis with their roots (2,3). This is because orchids lack a food reserve (endosperm), requiring mycorrhizal fungi, known as Orchid Mycorrhizal Fungi (OMF), to aid nutrient absorption and seed germination (4). Mycorrhizal fungi supply essential nutrients such as nitrogen and phosphorus to the plant and facilitate the germination of orchid seeds, which naturally lack food reserves (5).

One group of fungi commonly found on orchid roots is *Rhizoctonia*-like fungi, which are characterized by white to cream-colored colonies with perpendicularly branching, septate hyphae (6). In addition to providing a nutrient supply to orchid seeds, this group of OMFs can also increase *Dendrobium's* resistance to pathogens. Research by (7) explains that *Rhizoctonia* mycorrhizal can induce lignification in orchid root and leaf tissue, thereby strengthening their anatomical structure. This increased lignification contributes to increased plant resistance to various pathogens, including Odontoglossum ringspot virus (ORSV). Thus, *Rhizoctonia* mycorrhizal not only serve as nutrient providers during the vegetative growth phase but also function as biological agents that support the orchid's natural defense mechanisms.

The genus *Dendrobium* exhibits considerable root structural diversity across its species, including *Dendrobium spectabile*, *Dendrobium lineale*, *Dendrobium aphyllum*, *Dendrobium discolor*, and *Dendrobium fimbriatum*. These variations in root anatomy have the potential to create differences in the types and characteristics of *Rhizoctonia* mycorrhizae that form symbiotic associations within each species. This can be reflected in variations in colony morphological characteristics, such as color, growth pattern, and hyphal shape. Therefore, more in-depth observations are needed to understand the differences in mycorrhizal characteristics between species within the genus *Dendrobium* (8). Through detailed morphological and anatomical identification, this study is expected to provide comprehensive scientific information regarding the differences in *Rhizoctonia* mycorrhizal characteristics and support the development of microbiology-based orchid cultivation and conservation technologies.

Material and Methods

The materials used were Potato Dextrose Agar (PDA), roots of *D. spectabile*, *D. lineale*, *D. aphyllum*, *D. discolor*, and *D. fimbriatum*, Safranin O, and alcohol. Samples were taken from healthy roots according to the method of (9). Isolation of *Rhizoctonia*-like mycorrhiza was carried out by cutting the tip of a healthy *Dendrobium* sp. root with a thickness of 3 mm in cross-section. Root segments were placed on potato dextrose agar (PDA) in a Petri dish with a pH of 7.2 that had been sterilized at 121^0C in an autoclave for 15 minutes. Sterilization of *Dendrobium* sp. root samples was not carried out because it could kill other endophytic fungi associated with orchid roots or those not associated with orchid roots (10). The fungi grown on PDA were subcultured again to obtain a single isolate with the white colony color criteria, which is a characteristic of the *Rhizoctonia* mycorrhizal group. Morphological characteristics were observed, namely, the color of the colony. At the same time, microscopic (anatomical) observations were the branching form. The number of nuclei in the cells was counted under a binocular microscope XSZ 107 BN/Oregon/Yazumi with a magnification of 400 times, with Safranin O staining and 90% alcohol, so that the hyphae and nuclei would be colored red (11). The observation method was used to identify the morphological results. In contrast, the quantitative approach was used to calculate the number of nuclei of *Rhizoctonia* mycorrhizal fungi cells from five species of *Dendrobium* sp. without statistical analysis of 150 samples.

Results and Discussion

The results of this study show that the morphological and anatomical characteristics of *Rhizoctonia* mycorrhizal fungi isolated from five species of *Dendrobium* orchids (*D. spectabile*, *D. lineale*, *D. aphyllum*, *D. discolor*, and *D. fimbriatum*) show different patterns in each colony (Figure 1).

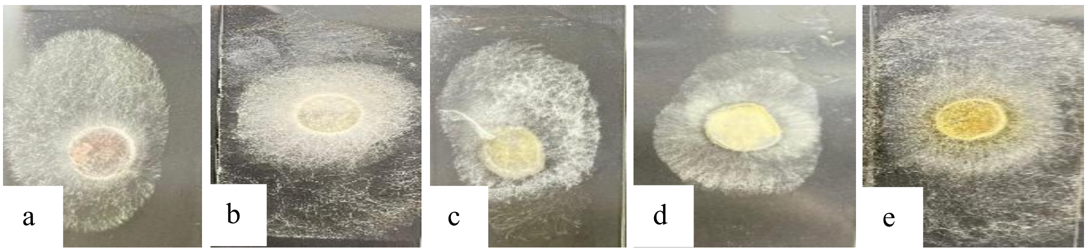

Figure 1. *Dendrobium spectabile* (a), *Dendrobium lineale* (b), *Dendrobium aphyllum* (c), *Dendrobium discolor* (d), and *Dendrobium fimbriatum* (e).

The fungal colony isolated from the roots of *D. spectabile* in the image shows a white-cream color with a slightly denser center. At the same time, the mycelium is evenly distributed and forms a smooth colony without sharp edges. This spread-out colony shape and pale color are common characteristics of mycorrhizal fungi of the *Rhizoctonia*-like group that are often found on orchid roots. After subculturing, hyphal growth in the selected colonies demonstrated characteristics more consistent with the morphology of *Rhizoctonia* mycorrhizal. Cultivated on glass, the hyphae appeared more regular, smooth, and spread out in a radial pattern from the inoculation point. The mycelium was white to pale cream in color, with no prominent sporulation structures, indicating that the subculturing successfully reduced the presence of contaminating fungi previously present in the initial culture.

On glass, the mycelium appeared thinner and more evenly distributed due to its two-dimensional growth, allowing for clearer hyphal branching. This smooth, highly branched, and spread-out appearance of the mycelium supports the isolate's ability to grow well outside of root tissue. This pattern aligns with reports on orchid mycorrhizal, which describe *Rhizoctonia*-like fungi producing thin, highly branched mycelium when grown on flat surfaces (12). When observing the hyphae on the glass deck, the mycelium appeared yellowish-white with a growth pattern that spread radially or converged from a single point. This pattern indicates the typical growth of mycorrhizal fungi from the *Rhizoctonia* mycorrhizal group, which have thin, branching, and evenly distributed hyphae. The radial pattern on the glass deck reinforces the suspicion that the primary isolate originated from a mycorrhizal fungus living in symbiosis with orchid roots, as this growth pattern is commonly reported for *Rhizoctonia* sp. when observed microscopically.

In the initial culture of the isolate from *D. fimbriatum* roots, the fungal colony appeared white-grayish with a cottony texture, then the center began to darken as the mycelium aged. After subculturing, the colony growth pattern became clearer: the center of the colony appeared dense and dark, while the edges were lighter and smoother, indicating the presence of mature mycelium in the center and young, actively growing mycelium at the edges. This radial growth pattern is a common characteristic of *Rhizoctonia* mycorrhizal of orchids, as explained by (12) that orchid mycorrhizal isolates usually form white colonies initially and then darken in the center as the mycelium matures. Overall, from the morphological observations of *Rhizoctonia* mycorrhizal colonies from five orchid species of *Dendrobium* sp., the colony color, colony shape, and growth pattern can be seen in Table 1 below.

Table 1. Morphological characteristics of *Rhizoctonia* mycorrhizal colonies from five species of *Dendrobium* sp.

Species	Colony Color	Colony form
D. spectabile	Bright creamy white	Circular
D. lineale	Creamy white	Circular regularly
D. aphyllum	Grayish white	The colony is not very firm
D. discolor	Pale pink with thin white hyphae	Imperfectly circular
D. fimbriatum	Dark gray, bright white edges	Circular clear

Based on Table 1, all *Rhizoctonia* mycorrhizal isolates from five *Dendrobium* species exhibited a similar circular colony growth pattern, but with variations in color and colony shape. Isolates from *D. spectabile* and *D. lineale* were creamy white with a regular circular shape, indicating stable growth activity. Meanwhile, isolates from *D. aphyllum* appeared grayish white with less defined colonies, indicating slower growth. Isolates from *D. discolor* were characterized by a pale pink color and imperfectly circular colony shapes. In contrast, isolates from *D. fimbriatum* had dark grayish centers and bright white edges with a clear circular shape, indicating more mature colonies. These variations in colony color and shape do not reflect differences in fungal species, but are more influenced by culture age, growth phase, and physiological conditions of the fungus. This is in accordance with reports by (13) and (14), which stated that changes in *Rhizoctonia* colony morphology are determined more by physiological factors than genetic factors.

Microscopic observations were conducted to determine the hyphal structure of *Rhizoctonia* mycorrhizal isolated from five *Dendrobium* sp. species. Observations were conducted using a light microscope after staining with Safranin O due to limitations in our laboratory, which cannot use DAPI (4′,6-diamidino-2-phenylindole). Observations focused on right-angled branching, the presence of septa, and the number of cell nuclei to identify the characteristics of *Rhizoctonia* mycorrhizae. Of the five *Dendrobium* orchids explored, it turned out to produce several different hyphal forms and numbers of nuclei (Figure 2).

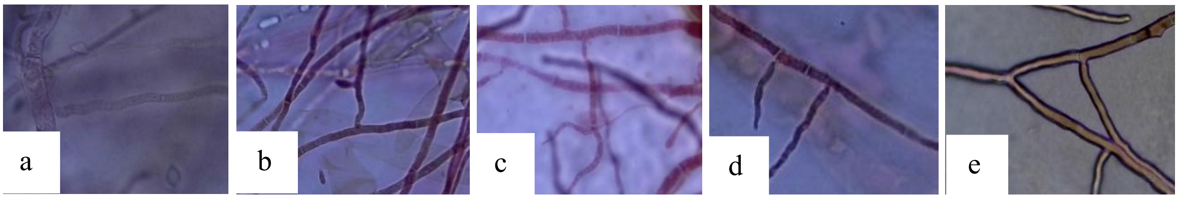

Figure 2. Percabangan siku-siku *Dendrobium spectabile* (a), *Dendrobium lineale* (b), *Dendrobium aphyllum* (c), *Dendrobium discolor* (d), dan *Dendrobium fimbriatum* (e).

Observations in Figure 2, conducted through root isolation, macroscopic (colony morphology), and microscopic (hyphal anatomy) measurements of each root of D. spectabile, D. lineale, D. aphyllum, D. discolor, and D. fimbriatum, show the presence of angled hyphae with different hyphal structures between one isolate and another. These data support the hypothesis that there are significant differences in morphological characteristics (colony color, colony shape, growth pattern) and anatomy (hyphal shape, number of cell nuclei) between species, despite their general similarity as active mycorrhizal fungi. These differences are likely influenced by fungal adaptation to different host species, growth phases, and physiological conditions.

Further observations were made on the number of hyphal cell nuclei to determine the physiological characteristics of each *Rhizoctonia* mycorrhizal isolate from five *Dendrobium* species. According to (15), *Rhizoctonia* species have different numbers of nuclei. Classification based on nuclei shows that some species have one nucleus (uninucleate), two nuclei (binucleate), and more than two nuclei (multinucleate). This data serves as a basis for identifying the physiological tendencies of fungi in each host species, especially their pathogenicity to plants (16). There are genetic differences between *Rhizoctonia* spp. groups because the multinucleate and binucleate groups have evolutionary and physiological differences: the multinucleate *Rhizoctonia* group is generally more pathogenic and aggressive (15,17) while the binucleate *Rhizoctonia* group is often less virulent or even antagonistic to other pathogens (18). The results of observations on the number of hyphal cell nuclei for all isolates are presented in Table 2.

Table 2. Number of *Rhizoctonia* mycorrhizae cell nuclei

V (5 isolates)	Total 25 Cell Nuclei			Percentage (%)	Description
	1	2	>2		
D. spectabile	3	16	6	72,7%	binucleate
D. lineale	3	19	3	76%	binucleate
D. aphyllum	2	20	3	80%	binucleate
D. discolor	6	11	8	44%	binucleate
D. fimbriatum	3	15	7	60%	binucleate

The observations in Table 2 show that the composition of the number of nuclei of *Rhizoctonia* mycorrhizal hyphae in five *Dendrobium* sp. species has quite clear differences. Isolates from *D. aphyllum* have the highest percentage of binucleate cells, namely 80%, so they can be categorized as isolates with the most stable physiological characteristics. The dominance of these multiple nuclei indicates that the fungus in this species has good symbiotic abilities, because binucleate hyphae are the main characteristic of the binucleate *Rhizoctonia* (BNR) group, which plays an important role in the formation of peloton in orchid root cells. This finding is in line with (19), who stated that binucleate *Rhizoctonia* is the most common and most effective type of mycorrhizal in supporting orchid growth.

Isolates from *D. lineale* (76%) and *D. spectabile* (72.7%) also showed high binucleate percentages, thus being considered to have good symbiotic abilities. These two isolates had a nuclear composition pattern similar to *D. aphyllum*, although their stability was slightly lower. Meanwhile, the isolate from *D. fimbriatum* had a binucleate value of 60%, thus categorized as moderate. This percentage still indicates the dominance of multiple nuclei. Still, the greater proportion of uninucleates and multinucleates compared to the best isolate indicates that nucleus formation in this isolate was not as strong and effective as the previous three isolates. This difference is thought to be related to root conditions during isolation or the level of physiological stress of the plant, as explained by (5), which states that the conditions of the orchid root microhabitat can influence variations in the number of nuclei in Rhizoctonia.

The most striking difference was seen in the *D. discolor* isolate with a binucleate percentage of only 44%, making it the isolate with the lowest nuclear stability. The low dominance of multiple nuclei indicates that this fungus likely has a less harmonious symbiotic relationship with its host or is in suboptimal root conditions. The high number of uninucleate and multinucleate cells in this isolate also indicates an imbalance in nuclear division within the hyphae, which can affect the fungus's ability to form a peloton structure. This is consistent with the findings of (6), who stated that the BNR group is the most stable type of mycorrhizae and plays the most important role in the formation of the functional peloton in *Dendrobium* roots. Thus, isolates from *D. aphyllum* can be considered the most promising as functional mycorrhizae, while isolates from *D. discolor* have the lowest potential in this study.

The implications of the findings of this study are the utilization of mycorrhizae from the Rhizoctonia mycorrhizae group obtained from the roots of *Dendrobium* orchid species as raw materials for plant growth-promoting fungi (PGPF), which can function in: [1] increasing growth, helping nutrient absorption, producing plant hormones, and fertilizing the soil (20). [2] protecting plants: functioning as bio-pesticides that help plants fight diseases, pathogenic fungi, and pests, and [3] as sustainable agriculture: Reducing dependence on chemical fertilizers and pesticides (21).

Conclusion

Based on the results of research on the morphological and anatomical identification of OMF isolated from five species of *Dendrobium* sp. orchids (*D. spectabile*, *D. lineale*, *D. aphyllum*, *D. discolor*, and *D. fimbriatum*), the research shows the morphological character of OMF with clear variations, especially in the color of the colony, which varies from white, cream, pale pink, to grayish, and the radial hyphal growth pattern typical of *Rhizoctonia* species. Although there are differences between isolates, all OMF colonies display the general characteristics of *Rhizoctonia* mycorrhizal, which have 2 nuclei (binucleate). *D. aphyllum* has the highest percentage of binucleate cells (80%), so they are considered the most stable and have strong potential as functional mycorrhizae. In contrast, *D. discolor* had the lowest binucleate value (44%).

Conflict of Interest

The author should declare all relationships, financial, commercial, or otherwise, the academic community might perceive that as representing a potential conflict of interest. If there are no such relationships, the author can state “All authors declare no conflicts of interest” in this section.

References

[1] Demena M, Raunsay EK, Agustini V. Characters of epiphyte and terrestrial orchid habitats in Kantumilena Village, Yokari District, Jayapura Regency. J Kehut Papuasia. 2020;6(1). doi:10.46703/jurnalpapuasia.Vol6.Iss1.198. [Indonesian]

[2] Attri KL. Studies on mycorrhizal associations in orchids. Asian J Biol Life Sci. 2023;12(1):179–186. doi:10.5530/ajbls.2023.12.24.

[3] Salazar J, Pomavila M, Pollard AT, Chica EJ, Pena DF. Endophytic fungi associated with roots of epiphytic orchids in two Andean forests in southern Ecuador and their role in germination. Lankesteriana. 2020;20(1):37–52.

[4] Jacquemyn H, Waud M, Brys R, Lallemand F, Courty PE, Robionek A, et al. Mycorrhizal associations and trophic modes in coexisting orchids: an ecological continuum between auto- and mixotrophy. Front Plant Sci. 2017;8:1497.

[5] Tsulsiyah B, Farida T, Sutra CL, Semiarti E. Important role of mycorrhiza for seed germination and growth of Dendrobium orchids. J Trop Biodivers Biotechnol. 2021;6(2). doi:10.22146/JTBB.60805.

[6] Soelistijono R, Utami DS, Daryanti, Faizin M, Dian R. Short communication: Characterization of rhizoctonia-like mycorrhizae associated with five Dendrobium species in Java, Indonesia. Biodiversitas. 2020;21(3):1007–1011. doi:10.13057/biodiv/d210321.

[7] Mahfut, Hidayat MM, Arifannisa SJ. Study of orchid resistance induction using Rhizoctonia against ORSV infection based on anatomical characters of roots and leaves. Asian J Plant Sci. 2023;22(2):239–249. doi:10.3923/ajps.2023.239.249.

[8] Arsitalia M, Wahyudi, Irawan B, Mahfut. Identification of orchid mycorrhiza in roots of Dendrobium nobile. Florea J Biol Dan Pembelajarannya. 2023;10(2):60–65. doi:10.25273/florea.v10i2.21187. [Indonesian]

[9] Zettler LW, Corey LL. Orchid mycorrhizal fungi: isolation and identification techniques. Methods Mol Biol. 2018. doi:10.1007/978-1-4939-7771-0_2.

[10] Shah S, Shah B, Sharma R, Rekawad B, Shouce YS, Sharma J, et al. Colonization with non-mycorrhizal culturable endophytic fungi enhances orchid growth and indole acetic acid production. BMC Microbiol. 2022;22:101. doi:10.1186/s12866-022-02507-z.

[11] Ajayi-Oyetunde OO, Bradley CA. Identification and characterization of Rhizoctonia species. Plant Health Instr. 2020.

[12] Bhuiyan M, Hossain M, Hossain KS, Islam MN. Isolation and identification of mycorrhizal fungus from an epiphytic orchid (Rhynchostylis retusa L. Bl.). Bangladesh J Bot. 2021;50(1). doi:10.3329/bjb.v50i1.52675.

[13] Soelistijono R, Daryanti, Rakhmawati, Rianto PA, Utomo H. Utilization of Rhizoctonia mycorrhizae for orchid late blight control in sustainable agriculture. Biosaintifika. 2024;16(1):1–9.

[14] Das S, Plyler-Harveson T, Santra DK, Maharjan B, Nielson KA, Harveson RM. A longitudinal study on morpho-genetic diversity of pathogenic Rhizoctonia solani from sugar beet and dry beans of western Nebraska. BMC Microbiol. 2020;20(1). doi:10.1186/s12866-020-02026-9.

[15] Ajayi-Oyetunde OO, Bradley CA. Rhizoctonia solani: taxonomy, population biology and management of rhizoctonia seedling disease of soybean. Plant Pathol. 2018;67:3–17. doi:10.1111/ppa.12733.

[16] Razali NM, Hisham SN, Kumar IS, Shukla RN, Lee M, Bakar MFA, et al. Comparative genomics: insights on the pathogenicity and lifestyle of Rhizoctonia solani. Int J Mol Sci. 2021;22(4):2183. doi:10.3390/ijms22042183.

[17] Moliszewska E, Maculewicz D, Stępniewska H. Characterization of three-nucleate Rhizoctonia AG-E based on their morphology and phylogeny. Sci Rep. 2023;13:17328.

[18] Hossain MM. Morpho-molecular characterization of Ceratobasidium sp.: a mycorrhizal fungus isolated from a rare epiphytic orchid Gastrochilus calceolaris (J.E. Sm.) D. Don. Bangladesh J Plant Taxon. 2019;26(2):249–257. doi:10.3329/bjpt.v26i2.44584.

[19] Chauhan P, Attri LK. Mycorrhizal associations in orchids: a review. Asian J Biol Life Sci. 2024;13(2):278–286. doi:10.5530/ajbls.2024.13.36.

[20] Li T, Yang E, Wu S, Selosse MC, Gao J. Progress and prospects of mycorrhizal fungal diversity in orchids. Front Plant Sci. 2021;12:646325.

[21] Shreya D, Mohanty S, Sahu G, Sarkar S. Sustainable agriculture: a path towards a better future. Food Sci Rep. 2020;1:22–25.