Isolation and Identification of *Rhizoctonia* Associated with *Phalaenopsis amabilis* (L.) Blume Roots

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ABSTRACT

Most of orchid mycorrhizal fungi are from *Rhizoctonia* genus and these fungi have known can enhance the growth of orchid. This study aimed to isolate and identify *Rhizoctonia* associated with *Phalaenopsis amabilis* roots. Isolation of *Rhizoctonia* from healthy roots was carried out using a modification of Yuan method. Identification was based on the macroscopic and microscopic characteristics. Nuclei were stained with safranin O and KOH. Four *Rhizoctonia* isolates isolated from root of *Ph. amabilis* were identified as *Ceratorhiza* sp1, *Ceratorhiza* sp2, *Epulorhiza repens*, and *Moniliopsis* sp. Nuclear staining revealed that *Ceratorhiza* sp1, *Ceratorhiza* sp2 and *Epulorhiza repens* were binucleate and *Moniliopsis* sp. was multinucleate.

Key word : *Rhizoctonia*, *Phalaenopsis amabilis*, isolation, identification, orchid

INTRODUCTION

Phalaenopsis amabilis (L.) Blume or moon orchid is one of Indonesia native orchid. This orchid was determined as Indonesian National Flower called Puspa Pesona. Naturally, all orchids are associated with endophytic fungi. All orchids utilize endophytic fungi to initiate seed germination, to enhance growth and to protect orchid from pathogen (Batty *et al*., 2001; Chang, 2007; Dearnaley, 2007; Lee, 2002).

Based on earlier studies, it was known that most isolated fungi from orchid roots were classified as *Rhizoctonia* or *Rhizoctonia*-like fungi (Otero *et al*., 2002; Shimura *et al*., 2009). *Rhizoctonia*-like fungi included the anamorphic genera *Ceratorhiza*, *Epulorhiza*, *Moniliopsis*, and *Rhizoctonia* and the teleomorphic genera were *Ceratobasidium*, *Sebacina*, *Tulasnella* and *Thanatephorus* (Dearnaley, 2007; Mursidawati, 2007; Rasmussen, 2002)

Research by Wu *et al*. (2010) showed that inoculation of *Rhizoctonia* spp. could enhance fresh biomass, plant height and leaf number of *Cymbidium goeringii* seedlings. Shan *et al*. (2002) also reported that *Epulorhiza* isolates were strongly stimulated the germination and development of three orchid's species (*Arundina chinensis*, *Spathoglottis pubescens* and *Spiranthes hongkongensis*).

In Indonesia, research on *Rhizoctonia* has been done by Sudanta and Abadi (2006) and Irawati (2005) on *Vanilla*. There are still limited studies on the association between *Rhizoctonia* and *Phalaenopsis* roots, therefore, it is necessary to further examine the isolation and identification of *Rhizoctonia* from *Ph. amabilis* roots. This study aimed to isolate and identify of *Rhizoctonia* associated with *Ph.amabilis* roots.

MATERIAL AND METHODS

Healthy *Ph. amabilis* roots were collected from Mekarlestari Orchid Nursery Yogyakarta and Handoyo Budi Orchid Nursery Malang. Isolation and identification was done at Laboratory of Plant Taxonomy, Faculty of Biology, Gadjah Mada University from October 2010 – January 2011.

Rhizoctonia isolation and culture

Healthy roots were rinsed with tap water slightly, immersed in ethanol 75% for 40s, immersed in sodium hypochlorite 4% for 10 min and finally rinsed in sterile distilled water with three times. Roots were cut into 0.5-1 cm length section and transferred to a plate with PDA medium supplemented with chloramphenicol to avoid bacteria growth. Plates were sealed with Parafilm to avoid desiccation and cultured at room temperature in dark. Hyphae emerging from segments were subcultured onto fresh PDA for purification (Yuan, 2009)

Identification

The identification of the isolates was based on the characteristic of the hyphal branching and septation pattern. Hyphal and moniliod cells were measured. The number of nuclei per cell was determined by examination after staining with safranin O and KOH. Literatures used in the fungal identification were (Athipunyakom *et al*., 2004; Shan *et al*., 2002; Currah and Zelmer, 1992).

RESULT AND DISCUSSION

Four isolates were recovered from *Ph. amabilis* roots. Based on morphological characteristics, four isolates showed *Rhizoctonia* characters such as branching near the distal septum in young vegetative hyphae, formation of a septum in the branch of near of the point of origin, constriction of branch hyphae at the point of origin, right angle (90º) branches of hyphae and produced monillioid cells on old culture. Further identification showed that the isolates were identified as *Ceratorhiza* sp1, *Ceratorhiza* sp2, *Epulorhiza repens*, and *Moniliopsis*. Nuclear staining revealed that *Ceratorhiza* sp1, *Ceratorhiza* sp2 and *Epulorhiza repens* were binucleate and *Moniliopsis* sp. was multinucleate. Four isolates are described below :

Ceratorhiza sp1

Host : Healthy roots of *Ph. amabilis* collected from Mekarlestari orchid nursery, Yogyakarta

On PDA, colony grew rapidly, 9 cm in diameter after 4 days incubation at room temperature, white when young turned to white to cream at maturity (Figure 1a). Observation under microscope showed that fungi produced septa and branched hyphae. Branches arise in right angles (90º) from the main hyphae (Figure 1b). Hyphae was Hyaline on color, with 2.5 – 7.5 µm in width. On mature colony (>7 days) hyphae produced monilliod cells. The monillioid cells were ellipsoidal or elongate barrel shape, the widht and the length were 5 – 12.5 x 20.0 – 50.0 µm (Figure 1c). Each cell of the hyphae and monillioid cells contained two nuclei or binucleate (Figure 1d).

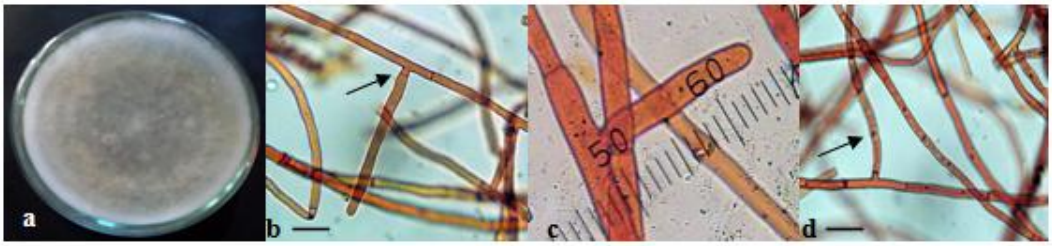

Figure 1. *Ceratorhiza* sp1 (a) colony on PDA 7 days, (b) 90º branched hyphae (arrow head), (c) monillioid cell and (d), binucleate hyphae (arrow head), Bar 10 µm

According to characteristic described before, the isolate have similar characteristics to the genus *Ceratorhiza* sp. that has been described by Currah and Zelmer (1992) such as colony color, aerial hyphae widht, branched hyphae and the number of nuclei. Athipunyakom *et al*. (2004) reported that one of the most abundant mycorrhiza associated with orchid is *Ceratorhiza* sp.

***Ceratorhiza* sp2**

Host : Healthy roots of *Ph. amabilis* collected from Handoyo Budi orchid nursery, Malang

On PDA, colony grew rapidly, 6.91 cm in diameter after 4 days incubation at room temperature, white when young turned to white to cream at maturity (Figure 2a). Observation under microscope showed that fungi produced septa and branched hyphae. Branches arise in right angles (90º) from the main hyphae (Figure 2b). Hyphae was Hyaline on color, with 5 µm in width. On mature colony (>7 days) hyphae produced monilliod cells. The monillioid cells were ellipsoidal or elongate barrel shape, the widht and the length were 7.5 – 10.0 x 20.0 – 25.0 µm (Figure 2c). Each cell of the hyphae and monillioid cells contained two nuclei or binucleate (Figure 2d).

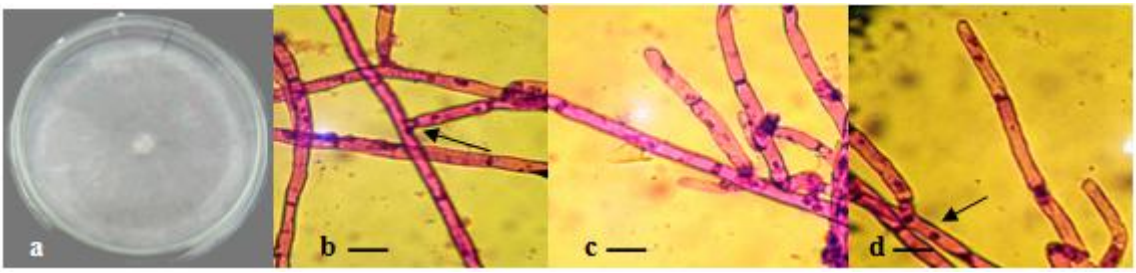

Figure 2. *Ceratorhiza* sp2. (a) colony on PDA 7 days, (b) 90º branched hyphae (arrow head), (c) monillioid cell and (d), binucleate hyphae (arrow head), Bar 10 µm

Based on the characteristic described before, the isolate showed similar characteristic to *Ceratorhiza* sp. by Shan *et al*. (2002) such as colony color, width and color hyphae, and dimension of monilioid cells, but different in colony growth rate. The growth rate of isolate was faster than *Ceratorhiza* sp. by Shan *et al*. (2002). Shan reported that the growth rate of *Ceratorhiza* sp. was 0.42-0.52 mm/h, but the isolate of this study was 0,72 mm/h. Currah (1992) reported that *Ceratorhiza* sp. had fast growth rate, furthermore Athipunyakom *et al.* (2004) reported that after 4-5 days incubation *Ceratorhiza* sp. growth could reach 9 cm in diameter and it was not differ much to the isolate of *Ceratorhiza* sp.2. Comparison based on the characteristic of *Ceratorhiza* sp.2. with *Ceratorhiza* sp. by Shan *et al.* (2002), Currah *et al*. (1992) and Athipunyakom *et al.* (2004), confirmed that the identity of isolate was*Ceratorhiza* sp.

Ceratorhiza sp. is an anamorphic genera for binucleate *Rhizoctonia* with dolipore septa and perforate parenthosomes, with telemorph *Ceratobasidium* (Shan *et al*., 2002; Garcia *et al*., 2006). *Ceratobasidium* spp. were known as pathogens of turfgrasses and cereals and had been reported as orchid endophytes in Australia, North America, and tropical Asia (Otero *et al.*,2002).

Epulorhiza repens

Host : Healthy roots of *Ph. amabilis* collected from Mekarlestari orchid nursery, Yogyakarta

On PDA, colony grew rapidly, 8.9 cm in diameter after 4 days incubation at room temperature, white to cream and sub merged (Figure 3a). Observation under microscope showed that the fungi produced septa and branched hyphae. Branches arise in right angles (90º) from the main hyphae (Figure 3b). Hyphae was hyaline on color, with 2,5 – 5 µm in

width. On mature colony (>7 days) hyphae produced monilliod cells. The monillioid cells were ellipsoidal to spherical shape, the width and the length were 10.0 – 15.0 x 15 – 25.0 µm (Figure 3c). Each cell of the hyphae and monillioid cells contained two nuclei or binucleate (Figure 3d).

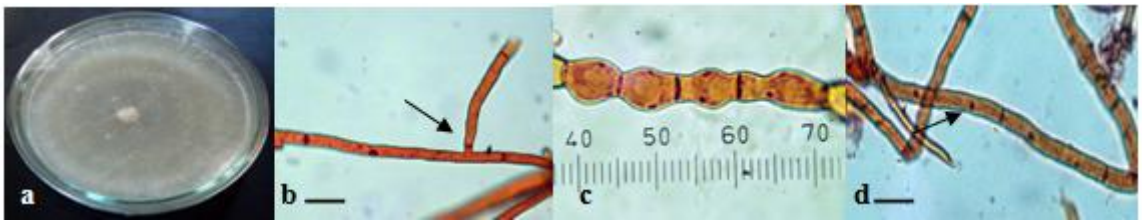

Figure 3. *E. repens* (a) colony on PDA 7 days, (b) 90º branched hyphae (arrow head), (c) monillioid cell and (d) binucleate hyphae (arrow head), Bar 10 µm

Currah and Zelmer (1992) reported that *Epulorhiza repens* produced cream-coloured colony and usually submerged, hyphae's colour was hyaline and monillioid cells ellipsoidal nearly spherical, these characteristics were similar to the characteristics of isolate described previously. Based on the comparison between characteristics of isolate and *E. repens* described by Currah and Zelmer (1992), it was confirmed that the identity of the isolate was *E. repens*, which is the anamorph of *Tulasnella calospora*.

The research carried out by Athipunyakom *et al*. (2004) found that there were several *E. repens* associated with *Spathoglottis plicata* in Chiang Mai and Chanthaburi. These fungi were tested to promote the germination and development of *S. plicata* seeds *in vitro*. The result showed that *E. repens* had the capability to stimulate growth and development of *S. plicata*.

***Moniliopsis* sp.**

Host : Healthy roots of *Ph. amabilis* collected from Mekarlestari orchid nursery, Yogyakarta

On PDA, colony had a moderately fast growth rate with 5.36 cm in diameter after 4 days incubation at room temperature, white to yellowish (Figure 4a) and turned to brownish with age. Observation under microscope showed that the fungi produced septa and branched hyphae. Branches arise in right angles (90º) from the main hyphae (Figure 4b). Hyphae was hyaline on color, with 5 – 7.5 µm in width. On mature colony (>7 days) of hyphae produced monilliod cells. The monillioid cells were barrel shaped, the width and the length were 10 x 20 – 25 µm (Figure 4c). Each cell of the hyphae and monillioid cells was multinucleate (Figure 4d).

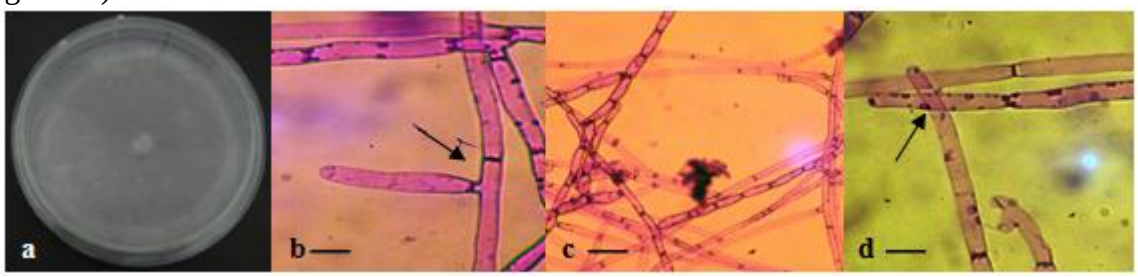

Figure 4. *Moniliopsis* sp. (a) colony on PDA 7 days, (b) 90º branched hyphae (arrow head), (c) monillioid cell and (d) multinucleate hyphae (arrow head), Bar 10 µm

Based on similar characteristic of the isolates to *Moniliopsis* sp. by Currah (1992), the isolate was identified as *Moniliopsis* sp. Athipunyakom *et al.*, (2004) stated that *Moniliopsis* sp. wasan anamorphic genera for multinucleate strains with perforate parenthosomes of *Thanatephorus* or *Waitea* telemorphs. *Rhizoctonia solani* (teleomorphs : *Thanatephorus*), the member of *Moniliopsis* sp., was known as important plant pathogens (Otero, *et al.*,2002)

CONCLUSION

Three genera from four isolates of *Rhizoctonia* fungi which were isolated from healthy *Phalaenopsis amabilis*'s roots, were identified as *Ceratorhiza* sp.1 and *Ceratorhiza* sp.2, *Epulorhiza repens* (binucleate *Rhizoctonia)*, and *Moniliopsis* sp. (multinucleate *Rhizoctonia*).

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