CENDAWAN ENDOFIT DI SEKITAR BANGUNAN KAMPUS: CATATAN DAN POTENSI BIOKONTROL

Endophytic Fungi Around Campus Building : Notes and Biocontrol Potency

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Abstrak:Cendawan endofit mengkolonisasi jaringan sehat tumbuhan tanpa menyebabkan kerusakan atau menghasilkan substansi yang menyebabkan infeksi pada jaringan inang. Penelitian mengenai cendawan endofit dan pemanfaatannya semakin banyak dilakukan dalam beberapa tahun terakhir di Indonesia. Namun, hingga saat ini informasi mengenai aspek tersebut di sekitaran bangunan kampus masih sangat terbatas. Tujuan dari penelitian ini adalah untuk mengisolasi cendawan endofit dari bebrapa tumbuhan Angiospermae di sekitaran bangunan kampus Institut Pertanian Bogor dan menguji kemampuannya sebagai agen biokontrol pada beberapa patogen tumbuhan. Sebanyak 9 isolat cendawan endofit diperoleh dari penelitian ini. Semua isolat menunjukkan karakter yang unik pada media PDA. Sebagian besar isolat memiliki aktivitas penghambatan terhadap patogen tanaman. AR1V1 dan menunjukkan aktivitas tertinggi terhadap Phythopthoracapsici sedangkan BWIV1 terhadap Fusarium oxisporumf. sp. cubense. Penelitian ini merupakan langkah awal untuk mengeksplorasi potensi cendawan endofit di sekitar bangunan kampus di masa mendatang

Kata kunci :Cendawan, Endofit, Bangunan kampus, Catatan, Potensi biokontrol

Abstract:Endophytic fungi occupied healthy plant tissues without destroying or producing substances which lead an infection to the host cell.Studies on

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endophytic fungi and its utilization have gained significance during therecent years in Indonesia. However, information provide in the term of institutional area are limited, and campus building is no exception. the goal of this study was to isolate endophytic fungi from some Angiosperms around IPB University Campus Building (IPBUCB) and testing their potential utilization as biocontrol of some plant pathogenic fungi. A total of 9 isolates of endophytic fungi obtained from this study. All isolates shown unique characteristics on PDA medium. Most of isolates have inhibition activity againtsplant pathogenic fungi. ARIV1 and ARIV2 were performed the highest (%) of inhibition of *Phythopthoracapsici*while BWIV1 in *Fusarium oxisporum*f. sp. cubense. This research is an early step to reveal the potential of endophytic fungi around campus building in the foreseeable future

Keywords: Endophytic, Fungi, Campus building, Notes, Biocontrol potency

1. Introduction

Fungal endophytes exist at whole or part of their life cycle inside the healthy tissues of plant host without causing any symptoms of disease (Wilson 1995). Endophytic fungi have been isolated from lower to higher plant groups (Petrini 1986; Petriniet al. 1992; Taylor et al. 1999; Arnold et al. 2000; Davis et al. 2003;), and wide range of plant habitats (Espinosa-Garcia and Langenheim 1990: Arnold et al. 2000: Kumaresan and Suryanarayanan 2001; Suryanarayananet al. 2003), even from bi/triparte symbiosis (lichens) (Petriniet al. 1990). Endophytic fungi colonize all parts of plant tissues including roots, stems, leaves, bark, floral organs and in some cases can affect both ecological and physiological processes of their host (Schulz et al. 1999).

Endophytic fungi provide variety of potential benefits to their plant host. Many researcher reported the potential fungal

endophyte, Kurose et al. (2012) reported that Colletotrichum, Pestalotiopsis, Phoma, Phomopsis, and Alternaria are potentially useful as a biological control agent of rust fungus on Fallopia japonica (Japanese knotweed), Hakkaret al. (2014) proved that Trichoderma asperellum can surpressed growth of Phytophthora palmivora, pathogenic fungi of Cacao in Indonesia. In addition, Hashiba and Narisawa (2005) reported that endophytic fungi (Heteronicumchaetospira) has ability to provide nitrogen for its host chinese cabbage and also suppress diseases caused by Pseudomonas syringaepv. macricola and Alternaria brassicae on host leaves. These endophytic fungi were found in the root and suppress the diseases by inducing systemic resistance against leaf diseases. Sun et al. (2011) isolated Phomachrysanthemicola from Suaedaspecies (plant) from soils with high salinity and high concentration ofirons. This fungi then used in marginal agriculture area. In West Java, Marianingsihet al. (2015) reported that 5 isolates of fungal endophyte from Ujung Kulon mangrove were able to enhance the growth of *Lycopersiconesculentum*.

Studies of endophytes from tropical area e.g. *Tripterygium* wilfordii(Kumar and Hyde 2004), coffee leaves (Santamaria and Bayman 2005), twigs and bark of *Populustremula*(Santamaria and Diez 2005), and *Zingiberaceae*(Putra et al. 2015) provide the high estimates of species diversity. Another reports indicated that more than 100 species of fungi may be associated with a single host

plant species (Arnold *et al.* 2000; Gou *et al.* 2000; Stone *et al.* 2000). Since endophytic fungi are still poorly known, especially in the tropics, current estimated numbers of fungal species are probably conservative (Gamboa*et al.* 2002). It is imply that tropical endophytes are an important component of undescribed fungal diversity especially from non temperate area.

In order to get the better understanding of endophytic fungi, exploration of fungal endophyte around us is needed, and campus area is no exception. Till time, infomation regarding fungal endophytes from plant around IPBUCB has never been reported before. Thus, the goal of this study was to isolate endophytic fungi from some Angiosperms around IPBUCB and testing their potential utilization as biocontrol of some plant pathogenic fungi.

2. Material and Methods

The study was conducted at Mycology Laboratory, Department of Biology, IPB University in April-May 2018. **Plant materials.** Healthy plant samples: *Amaranthus spinosus*L., *Averrhoa bilimbi*L., *Colocasia esculenta*(L.) Schott, and *Nephelium lappaceum* L.were collected around IPBUCB. Leaves, roots, rhizome, and stem were collected and labelled and put in an ice box and brought to the laboratory. The isolation was performed on the same day.

Isolation of Endophytic Fungi

Endophytic fungi were isolated according to Okane et al. (2008) with modification. Samples were rinsed gently in running water to remove dust and debris for several minutes. Surface sterilization wasperformed by submerged them in 70% ethanol for 1 minute, following 1% sodium hypochlorite (NaOCl) for 2 minutes. Each set of plant was rinsed by sterile distilled water (SDW) for 1 minute, twice. Samples then were dried with sterile paper towel for 6-12 hours. After drying, each sample then cut into 4 segments and put on the medium. Samples were put on potato dextrose agar (PDA) and incubated at 25°C for 2 weeks. Mycelia which growing from each plant segments was isolated and purified by hyphal tip method using fine tungsten needle in the same media.

Morphology Observation of Fungal Endophyte

Endophytic fungi were firstly grouped on the characteristics of their colony appearance on PDA such as: colony shape, color, elevation, texture, mycelia type, edges, density, and diameter. Both microscopic and molecular identification were not performed.

Antagonistic Test Against Plant Pathogenic Fungi

Phythopthora capsici and Fusarium oxysporum f. sp. cubense were used as plant pathogenic fungi in this study. Those fungal pathogen were provided by IPBCC (Culture Collection). Antagonistic tes was conducted using direct opposition method. Plant pathogeninoculated in pairs with pure endophytic fungi

isolate in PDA(using 9 cm of Petri dish) with a distance of 3 cm betwen fungi (Figure 1). All treatment were incubated at room temperature for 7 days.

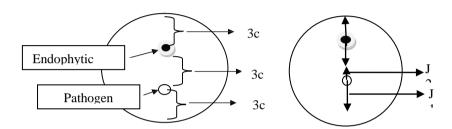


Figure 1. Schematic of fungal antagonistic test

Antagonistic activity observation was done referring to Fokkema (1973):

$$H = \frac{J1 - J2}{J1} \times 100\%$$

H= Antagonistic activity

J1= Pathogen growth to the edgenof Petri dish

J2= Pathogen growth to the endophytic fungi

3. Result and Discussion

Plant host were collected around IPBUCB, grew vigorously and healthy, so it was assumed that endophytic (not pathogenic) fungi were collected. As reported by Putra *et al.* (2015), plant host and ecosystem condition may affect both fungal community structure

and fungal life style (endophyte to pathogen). Endophytic fungi are normally found throughout parts of plant including leaves, stems, roots and also flowers. A total of 9 isolates (Table 1) were of collected. and most the fungi obtained from NepheliumlappaceumL .stem and leave were the most occupied organs in all host in contrast to the rhizome. Glienke-Blancoet al. (2002) proved that 81% of the total 433 endophytes isolated from leaves organs. It is implied that the occurance of endophytes concentrated in the leaves might influenced by the ability of the leaves to assistgrowth environment of endophytes. Furthermore, leaves are actively performsphotosynthesis so that nutrition is hugely available to the endophytic fungi.

Table 1. Pure Endophytic Fungi Obtained from This Study

No	Isolate code	Host
1	BTIV1	Colocasia esculenta
2	RTIV2	Colocasia esculenta
3	ARIV1	Nepheliumlappaceum
4	ARIV2	Nepheliumlappaceum
5	DRIV1	Nepheliumlappaceum
6	DRIV2	Nepheliumlappaceum
7	BWIV1	Averrhoa bilimbi
8	DBIV1	Amaranthus spinosus
9	BBIV1	Amaranthus spinosus

Another study confirmed that the leaves are hot spot for the diversity of endophytic fungi in the tropics (Arnold and Lutzoni,

2007). Sadeghi *et al.* (2019) reported that fungal endophyticcommunities living within the stem are assumed to be less stable than the ones inhabiting leaves, however the number of fungi in leaves may vary in the time being. It is explain why stem also poses same number of isolates with leaf in this study.

All isolates shown unique characteristics as described in Fig 2 and Table 3. Since both microscopic and molecular analysis were not applied in this study. Author consider not to put scientific identification only based on the colony characteristics. The identification can be performed at least by combining macroscopic and microscopic analysis, and fungi within species complex group require the molecular approach. Two isolates from stem and rhizome were collected from *Colocasia esculenta*(L.) Schottin this study. Some researchers reported the occurance of endophytuc fungi from Colocasia esculenta in Indonesia (Khastini 2018; Sulistiyono and Mahyuni 2019). However, those researchers acquired the plant host from natural ecosystem. Nepheliumlappaceum L.harboured 4 isolates of fungal endophyte in this study, emerged from root and leave. To the best of author's knowledge, there was no comprehensive report of endophytic fungi from NepheliumlappaceumL.in Indonesia. The only report was onlyabout bacterial isolation from this plant which performed by Suhandono*et al.* (2016). It is imply that there

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is still huge chance to explore diversity and potential utilization of endophytic fungi from *Nepheliumlappaceum* in Indonesia.

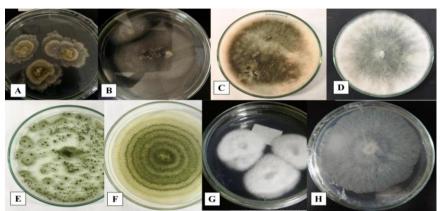


Figure 2. Some endophytic fungi obtained in this study. A: BTIV1; B:RTIV2; C:ARIV1; D:ARIV2;E:DRIV1; F:DRIV2; G:BWIV1; H:BBIV1.

Table 2. Characterictics of Endophytic Fungi Obtained After 14 days in PDA

Isolat Code	Colony Shape	Colour		Elevation	Texture	Mycelia	Edge	Density
		Above	Reverse	-		type		
BTIV1	Irregular	White	White	Convex with papilate surface	Velvety	Aerial	Filiform	Medium
RTIV2	Irregular	White to Cream	Cream to Yellow	Flat	Velvety	Aerial	Filiform	Dense
ARIV1	Filamentous	Dark Green	Dark Green	Flat	Cottony	Aerial	Entire	Dense
ARIV2	Filamentous	White	White to Cream	Flat	Cottony	Aerial	Entire	Dense
DRIV1	Circular	Light green	Green	Flat	Powdery	Aerial	Crenate	Spare
DRIV2	Irregular	Light green	Cream to green	Flat	Powdery	Aerial	Crenate	Dense
BWIV1	Circular	White	Grey to greenish	Convex with papilate surface	Cottony	Aerial	Undulate	Dense
DBIV1	Circular	Cream	Cream to yellow	Flat	Velvety	Aerial	Undulate	Dense
BBIV1	Circular	Dark Brown	Black	Flat	Velvety	Aerial	Undulate	Medium

Only one isolate recovered from *Averrhoa bilimbi* L. in this study. The assessment of isolation technique as well as media utilization likely need to be considered in the following research. Puta *et al.* (2015) used half strengh of malt extract agar (MEA) for endophytic fungi isolation and then PDA for purification purpose. However, many endophytic fungi also have been known as obligate mycobiont inside plant tissue. Hidayati*et al.* (2019) reported that *Penicilliumcitrinum* endophyte isolated from *Averrhoa bilimbi* L. has antibacterial activity against *Salmonella typhi* and *Staphylococcus aureus*. Two isolates from leaf and stem were collected from *Amaranthus spinosus*in this study. Scarce report ever found regarding endophytic fungi from this plant. The comperhensive report was provided by Sharma and Roy (2015), who reported that *Cladosporium* spp. which was found in all the plant parts of *Amaranthus spinosus* studied.

All of isolates have inhibition activity againtsplant pathogenic fungi, except DRIV2 (Table 3). ARIV1 and ARIV2 were performed the highest (%) of inhibition of *Phythopthoracapsici* while BWIV1 in *Fusarium oxisporum* sp. cubense treatment. Most endophyte isolates poses type B of fungal interactions regarding to inhibition of plant pathogen as described by Wheeler and Hocking (1993), while ARIV1 performed the F type. The B type interaction is mutual inhibition on contact or space between colonies is small (<2mm). The F type interaction is

inhibition of one species on contact or at a distance, the inhibitor species then continuing to grow at an unchanged rate through or over the inhibited colony (Wheeler and Hocking (1993). It is explain why at some treatment, fungal hypahe grew toward each other.

Phytophthora capsici and Fusarium oxysporumf. sp. cubenseare the main constraint in crops cultivation in Indonesia, especially on pepper and banana (Stover 1962; Wibowo et al. 2011; Wahyunoet al. 2016). Some attempts were performed to control these fungal pathogen using endophytic fungi as reported by (Ting 2010; Survanarayanet al. 2018), which reported that fungal endophytes haveremarkable potential as biocontrol agents and emphasize for obtaining more basic information the need endophyte biology, before they can be used effectively for biocontrol. Endophytes with potential as biocontrol agents are prevalent in plants in the rural areas, weeds and also medicinal plants (Rahman et al. 2009). Moreover, Yulianti (2016) stated that, as biocontol agents, endophytes could reduce plant damage by pathogens through induction for plant resistant mechanisms. In fact, endophytes can also act as biocontrol agents through antagonistic and competition interactions as proved in this study, despite of less-high inhibition provided by endophytic fungiin this study (Table 3).

Table 3. Antagonistic activity of endophytic fungi to plant fungal pathogen.

No	Isolate code	Pathogen	Inhibition (%)
1	BTIV1	Phythopthoracapsici	25,24
2	RTIV2	Phythopthoracapsici	19,5
3	ARIV1	Phythopthoracapsici	79
4	ARIV2	Phythopthoracapsici	79
5	DRIV1	Phythopthoracapsici	40,1
6	DRIV2	Phythopthoracapsici	_*
7	BWIV1	Fusarium oxysporumf. sp. cubense	66,67
8	DBIV1	Fusarium oxysporumf. sp. cubense	15,38
9	BBIV1	Fusarium oxysporumf. sp. cubense	56

^{-*:} Massively sporulated (antagonistic method was not appropriated)

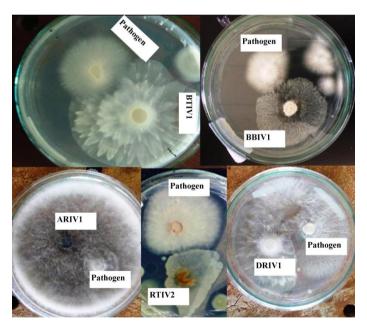


Figure 3. Antagonistic activity of some endophytic fungi to the fungal plant pathogen

4. Conclusions

A total of 9 isolates of endophytic fungi obtained from some Angiosperms around IPBUCB. All isolates shown unique characteristics on PDA. Most of isolates have inhibition activity againtsplantpathogenic fungi. ARIV1 and ARIV2 were performed the highest (%) of inhibition of *Phythopthoracapsici* while BWIV1 in *Fusarium oxisporum*f. sp. cubense. Following research is needed to reveal both the endophytic fungi diversity and potency from IPBUCB.

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