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Screening of endophytic fungi from tubers of *Dahlia variabilis*

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Researches on plant-derived endophytic fungi have grown in recent decades. *Dahlia variabilis* is one of natural products which potential as a source of antimicrobial agents. The purpose of this research was to isolate and characterize endophytic fungi from the tubers of *Dahlia variabilis* because derivation of bioactive compounds directly from the plants needs too many biomasses. Direct seeds method was used to isolate endophytic fungi from the samples. Samples were surface-sterilized by immersing in 70% ethanol, soaked in 15% peroxide acid, rinsed in sterile distilled water, and then put on the surface of Sabouraud-4% Dextrose Agar (SDA). Four isolates of endophytic fungi were isolated and characterized morphologically i.e., *Monilia* sp., *Aureobasidium* sp., *Moniliella* sp., and *Sporothrix* sp.

Key words: antimicrobial agents, *Dahlia variabilis*, endophytic fungi.

Introduction

Endophytic fungi are defined on healthy higher plant tissue by colonizing without causing any negative effects to their host. Endophytic fungi may offer either significant benefit to their host by producing secondary metabolites that provide protection, growth regulators, antimicrobials, antivirals, and insecticidals, or even mediate resistance to some types of abiotic stress (Chomcheon *et al*., 2009). Co-evolution or transfer genetic (genetic recombination) from the host to their endophytic fungi were assumed as the cause of those secondary metabolites production (Radji, 2005).

Methanolic extracts of the tubers of *Dahlia variabilis* have better activity to inhibit the growth of *Candida albicans* and *Microsporum gypseum* than

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ketoconazole at the same concentration (Octarina, 2010). Other researchers found that these extracts had the same analgetic activity with acidium acetylsalicylicum (Jannah, 2010). Hence, this study was conducted to isolate and characterize endophytic fungi from the tubers of *Dahlia variabilis*).

Material and methods

The tubers of *Dahlia variabilis* were collected from Padang Luar, West Sumatera randomly. The samples have different color of flower i.e., orange, yellowish, maroon, and purple. The samples were washed in running tap water and surface-sterilized by immersing in 70% ethanol for 3 minutes, then soaked in 15% peroxide acid for 5 minutes and again transferred to 70% ethanol for 1 minute, then rinsed in sterile distilled water, and surface-dried with sterile filter paper. To confirm that the surface sterilization was successful, aliquots of the sterile distilled water used in final rinse were inoculated onto the surface of SDA plates supplemented with Cefat® 30 µg/ml for 3 days (Ding *et al*., 2010).

Each of surface-sterilized samples was cut into fragments of approximately 1cm^2 and put onto the surface of SDA plates supplemented with Cefat® 30 µg/ml. The plates were incubated at room temperature for 5-7 days. The individual endophytic fungi colonies which growth on the media was placed to the new sterile SDA plates supplemented with Cefat® 30 µg/ml. The isolated endophytic fungi were then identified morphologically at Environment Microbiology Laboratory at Bandung Institute of Technology, Bandung, Indonesia.

Results

A total of 4 endophytic fungi isolated from tubers of *Dahlia variabilis* orange, yellowish, maroon, and purple, collected from Padang Luar, West Sumatera, Indonesia. All of the isolated endophytic fungi were identified morphologically at Environment Microbiology Laboratory at Bandung Institute of Technology, Bandung, Indonesia. Colony characteristic and microscopic appearance of endophytic fungi isolated from tubers of *Dahlia variabilis* orange, yellowish, maroon, and purple were shown at Fig. 1, Fig. 2, Fig. 3, and Fig. 4, respectively. Result of morphology characterization is shown at Table 1.

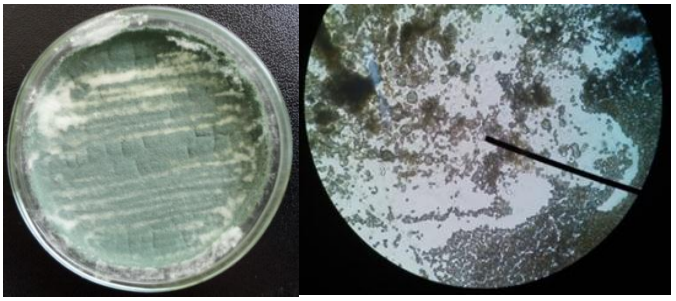

Fig. 1. Colony characteristic and microscopic appearance of *Monilia* sp. isolated from *Dahlia variabilis* orange.

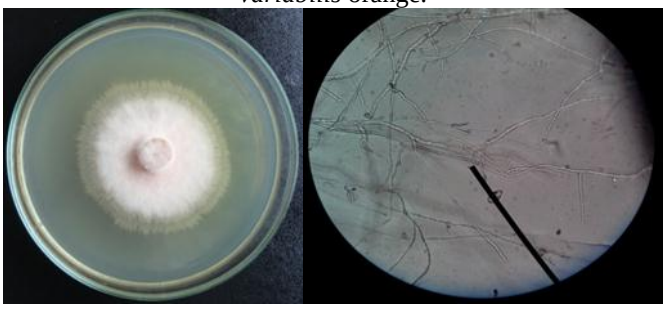

Fig. 2. Colony characteristic and microscopic appearance of *Aureobasidium* sp. isolated from *Dahlia variabilis* yellowish.

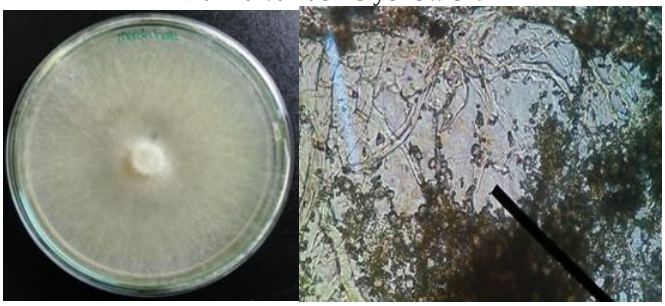

Fig. 3. Colony characteristic and microscopic appearance of *Moniliella* sp. isolated from *Dahlia variabilis* maroon.

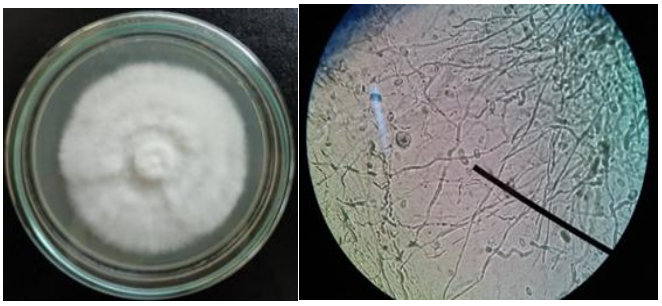

Fig. 4. Colony characteristic and microscopic appearance of *Sporotrix* sp. isolated from *Dahlia variabilis* purple.

Table 1. Morphological characteristic of endophytic fungi from the tubers of *Dahlia variabilis*

Characteristic	***Dahlia variabilis* orange**	***Dahlia variabilis* yellowish**	***Dahlia variabilis* maroon**	***Dahlia variabilis* Purple**
Color of colony	Green	White	Orange	White
Spore	+	+	+	+
Shape of spore	Amerospore	Amerospore	Amerospore	Amerospore
Color of mycelia	Hyaline	Hyaline	Hyaline	Hyaline
Color of conidia	Hyaline	Hyaline	Hyaline	Hyaline
Structure of conidia	Chain	Endoconidia	Chain	Single
Conidia from phialide	-	-	-	-
Thalus	-	-	-	-
Blastic (Holoblastic)	Spout	Enteroblastic	Spout	From narrow stake
Budding	+	+	+	+
Thicken of hyphae	-	-	+	-
Growth of colony	Fast	Medium	Very fast	Slow
Endophytic fungi	*Monilia* sp.	*Aureobasidium* sp.	*Moniliella* sp.	*Sporothrix* sp.

Discussion

Surface sterilization of the samples usually entails treating the samples with a strong oxidant, followed by a sterile rinse to remove residual sterilant. Peroxide acid (15%) is one of surface sterilant agent. Efficacy of surface sterilant often is improved by combining them with a wetting agent. Ethanol (70%) is the most commonly used wetting agent; it has limited antibiotic activity and should not be used alone as a surface disinfectant. The samples were rinsed in 70% ethanol after treatment for 1 minute to remove sterilant, and then rinsed in sterile distilled water to take a sterilization test. The sterile surface samples were inoculated onto surface of SDA plates supplemented with Cefat® 30 µg/ml to reduce contamination (Stone *et al*., 2004).

A total of 4 isolates of endophytic fungi were isolated from the tubers of *Dahlia variabilis* orange, yellowish, maroon, and purple, collected from Padang Luar, West Sumatera, i.e., *Monilia* sp., *Aureobasidium* sp., *Moniliella* sp., and *Sporothrix* sp., respectively. Colony color of *Monilia* sp. was green, shown at Fig. 1. The growth of this isolate was fast, with diameter of 5-7 cm after 5 days incubation. Colony color of *Aureobasidium* sp. was white, shown at Fig. 2. The growth of this isolate was medium, with diameter of 3-5 cm after 5 days incubation. Colony color of *Moniliella* sp. was orange, shown at Fig. 3. The growth of this isolate was very fast, spreading the plate in less than 24 hours incubation. Colony color of *Sporothrix* sp. was white, shown at Fig. 4. The growth of this isolate was slow, with diameter of 2-3 cm after 7 days

incubation. The growth optimization and metabolites production of each isolates is in progress at our laboratory.

Symbiosis endophytic fungi to *Dahlia variabilis* have been not reported from the electronic media yet, but endophytic fungi to family Compositae/Asteraceae have been already reported. Research about *Artemisia mongolica* gave result that endophytic fungus *Colletotriccum gloesopodioides* can produce a new antimicrobial metabolite, named colletotric acid which inhibited growth of *Bacillus subtilis*, *Staphylococcus aureus*, and *Sarcina lutea*, and pathogen fungus *Helminthosporium sativum*. Some kinds of genus Colletotriccum, plant fungi pathogens, have evoluted with interact endosymbiosis with some kinds of plants (Zou *et al*., 2000).

Endophytic fungus *Penicillium* sp. MH7 had been isolated from *Chrysanthemum coronarium*, potential to produce giberellin (Hamayun *et al*., 2010). A total of 39 endophytic fungi have been isolated from *Viguiera arenaria* and *Tithonia diversifolia*, which inhibit growth of leukimia cell, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Guimaraes *et al*., 2008). Endophytic fungus *Podospora* sp. which associated with *Laggera alata* have been isolated. The endophytic fungus produced two xanthones, sterigmatocystin and secosterigmatocystin, which showed mosquito larvicidal activities (Matasyoh *et al*., 2011).

Research about *Otanthus maritimus* gave result that endophytic fungus *Chaentomium* sp. produced a new tetrahydrofuran derivative (Aly *et al*., 2009). Endophytic fungus *Mycelia sterila*, which produced isocoumarin family, have been isolated from *Cirsium arvense* (Krohn *et al*., 2001). A total of 32 endophytic fungi have been isolated from *Smallanthus sandhifolius* which potential as resourceful producers of cytotoxic bioactive natural products (Gallo *et al*., 2009).

Conclusion

A total of 4 isolates of endophytic fungi were isolated from the tubers of *Dahlia variabilis* orange, yellowish, maroon, and purple from Padang Luar, West Sumatera, i.e., *Monilia* sp., *Aureobasidium* sp., *Moniliella* sp., and *Sporothrix* sp., respectively.

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