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Isolation and Selection of Potential Endophytic Fungi as Growth Inhibitors of Pathogenic *Fusarium oxysporum* Colonies on Black Pepper

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Abstract

Yellow disease in black pepper (*Piper nigrum* L.) is caused by plant parasitic nematodes and *F. oxysporum*. Wounds caused by parasitic nematodes create necrotic tissue in the roots, which facilitates infection by *F. oxysporum*. The use of endophytic microbes is an environment-friendly method that supports sustainable agriculture. This study aimed to identify potential endophytic isolates as biocontrol agents for *F. oxysporum*. Endophytes were isolated from the roots of black pepper plants of the Merapin and Lampung Daun Lebar (LDL) varieties at different cultivation sites. Endophytic fungi isolates were screened using the dual culture assay against the pathogenic fungus *F. oxysporum*. In this study, 98 endophytic fungal isolates were obtained, of which 13 isolates were found to have the potency to inhibit the growth of *F. oxysporum* mycelia by 7.78-50% on dual culture screening. The endophytic fungus *Gliocladium* sp. CMS8 can inhibit *F. oxysporum* mycelia by up to 50 %, making it a promising candidate for biological control of *F. oxysporum* in black pepper

Keywords: Competition, Root Endophyte, Lampung Daun Lebar (LDL), Merapin, *Piper nigrum*.

INTRODUCTION

F. oxysporum is one of the causal agent yellow disease in *P. nigrum* which infects the roots through natural wounds or infected by plant parasitic nematodes. This infection causes *P. nigrum* to be sensitive to drought and nutrient deficiencies, leading to yellow disease. This is a major disease affecting black pepper plantations on Bangka Island. Cultivated plant infections reached 20-60% in 2014 (Munif & Sulistiawati, 2014) and increased to 90% in 2022 (Ropalia *et al.*, 2022).

Biological control is an environment-friendly method that supports sustainable agriculture. Conceptually, biological control is a technique or method of suppressing pathogen activities that directly or indirectly cause diseases involving microorganisms. Types of microbes that can be used as biocontrols include soil microbes, microbes associated with plants (endophytic microbes), nodule microbes, rhizosphere microbes, rhizoplane microbes, and mycorrhizae.

Endophytic fungi have been reported to have the potential to suppress pathogen infections through various mechanisms. Endophytic fungi act as biocontrol through antibiosis, competition, and mycoparasitism (Bilanski & Kowalski, 2022), parasitism (Sergaran & Sathiavelu, 2019). *Trichoderma longibrachiatum* EF5 inhibits the mycelial growth of the pathogenic fungus in rice by producing aliphatic organic acids, aromatic nitroamino compounds, and volatile metabolites (Sornakili *et al.*, 2020). Endophytic fungi in bamboo roots have the potential to suppress *F. oxysporum* by 11.00 – 55.75% (Ropalia, 2017). The endophytic fungal isolates *Valsa friesii*, *Simplicillium lamellicola*, and *Cladorrhinum flexuosum* were able to inhibit the pathogenicity of *Fusarium graminearum* by 36-87% and Waitea circinata by 31-86% on wheat seedling leaves (Abaya *et al.*, 2021). The endophytic *Ramichloridium* sp. reduced the severity of anthracnose disease (*Colletotrichum gloeosporioides*) on *Euterpe precatoria* leaves by almost 100% (Peters *et al.* 2020). We examined the potential of endophytic fungi to control plant pathogens in previous studies. Therefore, in this study, we sought isolates that have the potential to inhibit the growth of *F. osxysporum*, which can be used as biocontrol agents in the future.

METHOD

Endophytic fungi were isolated from the roots of black pepper of Merapin and Lampung lampung Daun Lebar (LDL) varieties. The sampling sites were located in Beruas Village, Central Bangka Regency, Bangka Belitung Islands Province, Indonesia. The sample plants from different black pepper cultivation sites are

asymptomatic plant samples in asymptomatic and symptomatic plant populations. Root samples were collected from two plants at each site.

Isolation of Fungal Endophytes

The roots were washed from all particles adhering to their surfaces. Root bits (2 cm in length) were washed in running water for 1-2 hours. The samples were subjected to surface disinfection by immersion in NaOCl 2% for 2-3 minutes, alchohol 70% for 1-2 minutes, and finally rinsed in sterile aquadest three times. The samples were dried on sterile paper, cut shorter (5 mm) and both browned sides were cut. Five root bits were transferred to Petri dishes (diameter 10 cm) containing malt extract agar (MEA). Incubation was performed at room temperature for 1 month. As a control for surface sterilization, root bits were only traced on MEA media (without incubating the root bits) and incubated for 3 days at room temperature. Surface sterilization is successful if the media is not overgrowth by contaminating fungi or bacteria.

Dual culture screening

Before testing the antagonist, the pathogenicity of the isolates on rice seed germination was tested, and 61 nonpathogenic isolates were obtained. Then, an antagonistic test of endophytic fungi against *F. oxysporum* was carried out using the dual-culture method. One-week-old colonies of *F. oxysporum* and endophytic fungal isolates were cut using a cork borer with a 5 mm diameter. Endophytic fungi and *F. oxysporum* were grown together on PDA 3 cm apart in 10 cm diameter Petri dishes. As a control, two pieces of *F. oxysporum* colonies were grown without endophytic fungi.

The radius of the *F. oxysporum* and endophyte colonies was measured after the *F. oxysporum* mycelia that grew towards the edge of the Petri dish reached the outer side of the Petri dish. The inhibition of endophytic fungi against *F. oxysporum* miselia uses the following formula:

$$DH = \frac{R1-R2}{R2} x\ 100\%$$

DH = Endophytic fungi inhibition against *F. oxysporum* colony (%)
R1 = *F. oxysporum* colony radius towards the endophytic fungal colony
R2 = *F. oxysporum* colony radius away from the endophytic fungal colony

RESULTS AND DISCUSSION

Isolation of endophytic fungi from black pepper roots yielded 98 isolates, consisting of 52 isolates from the Merapin variety and 46 isolates from the LDL variety. Merapin obtained 23 and 29 isolates, and LDL variety obtained from 29 and 17 isolates, respectively, from samples from asymptomatic and symptomatic plant populations. The isolates were screened through a pathogenicity test on rice seed germination, and 61 non-pathogenic isolates were obtained, consisting of 32 isolates from the merapin variety and 29 isolates from the LDL variety.

Tabel 1. Number of isolated endophytic fungal isolates and nonpathogenic isolates

Variety	Sampling site[a]	Number of endophytes isolated results	Number of nonpathogenic endophytic fungi
Merapin	A	23	15
	B	29	17
Number of isolates		52	32
Lampung daun lebar (LDL)	A	29	19
	B	17	10
Number of isolates		46	29
Total number of isolates		98	61

[a] A = asymptomatic plant population, B = symptomatic plant population

The number of endophytic fungal isolates isolated from merapin was higher than that isolated from LDL. In merapin, the number of endophytic fungal isolates from the asymptomatic plant samples in the symptomatic plant populations was higher than that in asymptomatic plant populations, while the opposite occurred in the LDL variety. Differences in the varieties and sampling sites resulted in different numbers of endophytes. Previous studies have shown that the diversity and abundance of endophytes are greatly influenced by various factors, namely the ecosystem, location (Vaz *et al.* 2014), season (Lau *et al.* 2013), host type (Higgins *et al.* 2013), and tissue (Douanla-Meli *et al.* 2013).

Tabel 2. Endophytic fungi against *F. oxysporum* in dual-culture

Isolate code	*F. oxysporum* colony radius (cm)	Inhibitory (%)[a]
CMS2	0.93	37.78 b
CMS5	0.95	36.67 b
CMS8	0.75	50.00 a
CMS9	1.35	10.00 ef
CMS10	1.28	14.44 def
CMS14	1.27	15.57 def

CMI16	0.78	47.78 ab
CMI17	1.38	7.78 f
CMI24	1.13	24.44 c
CLS18	1.23	17.77 cde
CLS19	1.33	11.11 ef
CLS21	1.25	16.67 cde
CLI2	1.17	22.22 cd

[a] Numbers followed by the same letter in the same column show no significant difference in Duncan's test (α = 5%)

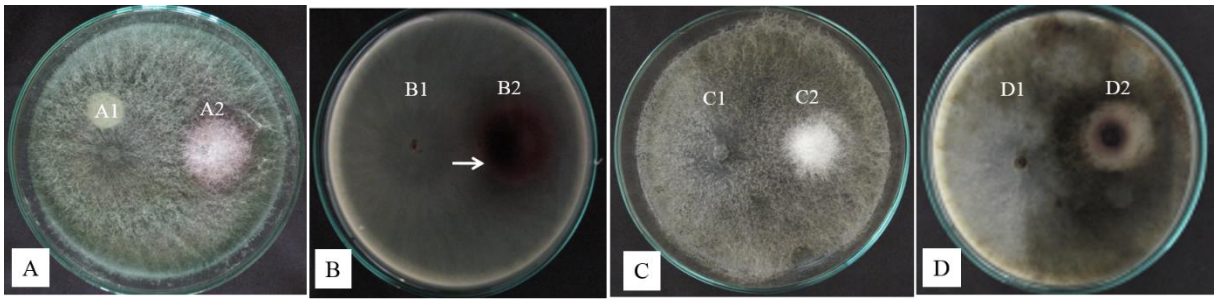

Figure 1. Endophyte antagonism against *F. oxysporum* in dual-culture, Antagonism of endophytic fungi CMS8: A) surface of the colony; B) bottom of the colony. antagonism of endophytic fungi CMI16; C) surface of the colony; D) bottom of the colony. A1 and B1) colonies of the fungal CMS8, C1, and D1) colonies of the endophytic fungi CMI16, and A2-D2) colonies of *F. oxysporum*. Pigmentation in the media around the *F. oxysporum* colony (arrows)

A dua culture assay of 61 endophytic fungal isolates to *F. oxysporum* obtained 13 antagonistic isolates that had an inhibition hambat of 7.78 - 50% (Table 2). The CMS8 isolate had the highest potential to inhibit *F. oxysporum* growth by up to 50%. The antagonistic mechanism of endophytic fungi against *F. oxysporum* involves competition for space and nutrients. The space colonization and competition for nutrients between endophytic fungi and *F. oxysporum* showed that endophytic fungi grew faster and filled the Petri dish space in the dual culture assay, so that the mycelial growth of pathogenic *F. oxysporum* was suppressed (Ropalia 2017). Four types of fungal endophyte and *Hymenoscyphus fraxineus* interactions in the dual culture assay were physical contact of mycelia, inhibition zone, overgrowth of fungal pathogen (*H. fraxineus*) colony by endophytes, and overgrowth of endophyte colonies by fungal pathogens (Bilanski & Kowalski 2022). The type of interaction of endophyte isoate CMS8 refers to the physical contact of mycelia. Pigmentation in the media around the *F. oxysporum* colony was presumably due to the exudate released by *F. oxysporum* and not from the endophytic fungus CMS8 isolate (Figure 2 B2). The

exudate secreted by endophytic fungi is usually followed by an inhibition zone, which acts as an antagonist that produces antimicrobials (Abaya *et al.* 2021).

The endophytic fungal isolate CMS8 had colony characteristics similar to those of *Trichoderma* sp. The microscopic characteristics of this isolate are hyaline and septal hyphae. Conidiophores are hyaline, erect, branched, and conidia are hyaline and globose. The spore mass clustered at the end of the fialids (Figure 2). The genus *Gliocladium* is characterized by branched conidiophores with verticillate or penicillate phialides that contain spore masses (clustered conidia) (Watanabe 2005). *Gliocladium catenulatum* is an endophyte from the *Theobroma cacao* plant that is antagonistic to *Crinipellis perniciosa* (Rubini *et al.* 2005).

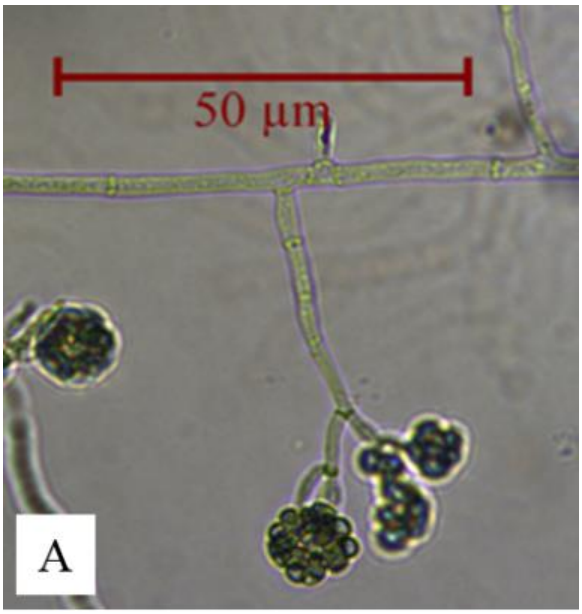

Figure 2. Microscopic morphology at 400 × magnification of the microscope compound

CONCLUSION

The isolation of endophytic fungi from pepper roots resulted in 98 isolates. of these, 61 were non-pathogenic. Thirteen isolates had the potential to inhibit the growth of *F. oxysporum* mycelia ranging from 7.78-50% in the dual culture method. The endophytic fungal isolate CMS8 (*Gliocladium* sp.) had the highest ability to inhibit the growth of *F. oxysporum* mycelia by 50% through a competition mechanism.

REFERENCE

Abaya A, Xue A, & Hsiang T. (2021). Selection and screening of fungal endophytes against wheat pathogens. *Biocontrol* 154: 104511. DOI: https://doi.org/10.1016/j.biocontrol.2020.104511

Bilanski P & Kowalski T. (2022). Fungal endophytes in *Fraxinus excelsior* petioles and their *in vitro* antagonistic potential against the ash dieback pathogen *Hymenoscyphus fraxineus. Microbiological Research* 257: 126961. DOI: https://doi.org/10.1016/j.micres.2022.126961

Douanla-Meli C, Langer E & Talontsi Mouafo F. (2013). Fungal endophyte diversity and community patterns in healthy and yellowing leaves of Citrus limon. *Fungal Ecology* 6(3): 212-222.

Higgins KL, Arnold AE, Coley PD & Kursar TA. (2014). Communities of fungal endophytes in tropical forest grasses: highly diverse host- and habitat generalists characterized by strong spatial structure. *Fungal Ecology* 8: 1-11.

Lau MK, Arnold AE & Johnson NC. (2013). Factors influencing communities of foliar fungal endophytes in riparian woody plants. *Fungal Ecology* 6(5): 365-378.

Munif A & Sulistiawati I. (2014). Pengelolaan penyakit kuning pada tanaman lada oleh petani di Wilayah Bangka. J. Fitofatologi Indonesia 10 (1): 8-16. DOI: 10.14692/jfi.10.1.8

Peters LP, Prado LS, Silva FIN, Souza FSC, Carvalho CM. (2020). Biological Control. Selection of endophytes as antagonists of *Colletotrichum gloeosporioides* in açaí palm. *Biocontrol* 150: 104350. DOI: https://doi.org/10.1016/j.biocontrol.2020.104350

Ropalia. (2017). Potensi Endofit akar bambu sebagai biokontrol patogen *Fusarium oxysporum* penyakit kuning tanaman lada. *Agrosainstek, 1*(2): 80-85.

Ropalia. (2022). Penyakit Utama Tanaman Lada di Kabupaten Bangka Selatan. *Agrosainstek* 6(1): 53-60. DOI: https://doi.org/10.33019/agrosainstek.v6i1.217

Rubini MR, Silva-Ribeiro RT, Pomella AWV, Maki CS, Araújo WL, dos Santos DR and Azevedo JL. (2005). Diversity of Endophytic Fungal Community of Cacao (*Theobroma cacao* L.) and Biological Control of Crinipellis perniciosa, causal agent of Witches' Broom Disease. *Int. J. Biol. Sci.* 1: 24-33.

Sergaran G & Sathiavelu M. (2019). Fungal Endophytes: A Potent Biocontrol Agent and a Bioactive Metabolites Reservoir. *Biocatalysis and Agricultural Biotechnology* 21 : 101284. DOI: https://doi.org/10.1016/j.bcab.2019.101284

Sornakili A, Thankappan S, Sridharan AP, Nithya P, Uthandi S. (2020). Antagonistic Fungal Endophytes and Their Metabolite-Mediated Interactions Against Phytopathogens in Rice. *Physiological and Molecular Plant Pathology*, 112: 101525

Vaz ABM, Fontenla S, Rocha FS, Brandão LR, Vieira MLA, de Garcia V, Góes-Neto A & Rosa CA. (2014). Fungal endophyte ß-diversity associated with *Myrtaceae* species in an Andean Patagonian forest (Argentina) and an Atlantic forest (Brazil). *Fungal Ecology*, 8: 28-36.

Watanabe T. (2010). Pictorial Altas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Spesies 2nd. New York (US). CRC Press LLC