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Colonization of *Trichoderma* Spp. in Rhizome of Five Zingiberaceous Species from North Sumatra, Indonesia

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Abstract

Zingiberaceae belongs to the medicinal plant family commonly utilized as herbal remedies by Indonesians to treat several health disorders. The study of its associative micro-organisms is gaining the focus of researchers, due to their diversity and potential application as biological control agents and novel compound producers. In this study, the diversity of *Trichoderma*, as fungal endophytes inhabiting the rhizome of wild Zingiberaceous species,was studied. Assemblage of *Trichoderma* species has been uncovered through molecular evidence based on ITS region, revealing their endophytic habits in Zingiberaceous species to be the most dominant soil mycoflora. The five Zingiberaceous species selected in this study were: *Alpinia* spp., *Amomum centrocephalum*, *Elettaria* spp., *Etlingera elatior*, and *Hedychium coronarium* sampled from Sibayak Forest, North Sumatera, Indonesia. The endophytic *Trichoderma* isolates were subjected to antagonistic assay against pathogenic bacteria and phytopathogenic fungi. Fifteen species of *Trichoderma* were identified on the basis of ITS-5.8S rDNA region in which were identified the species *T. afroharzianum*, *T. atroviride*, *T. brevicrassum*, *T. caribbaeum*, *T. dorotheae*, *T. gamsii*, *T. hamatum*, *T. hispanicum*, *T. koningii*, *T. koningiopsis*, *T. lixii*, *T. longibrachiatum*, *T. neokoningii*, *T. rifaii*, and *T. viridescens.* The majority of the isolated *Trichoderma* species were assigned as potential biocontrol agents against three phytopathogenic fungi, namely *Fusarium oxysporum*, *Ganoderma boninense* and *Rigidoporus lignosus*, while they were less effective towards pathogenic bacteria. The finding of endophytic *Trichoderma* spp. as the major mycoflora isolated from representative Zingiberaceous species in Sibayak Forest, suggests a potential for future application and study about inter-specific plant protection, pathogen inhibitors, and inducing plant systemic resistance.

Keywords: Antagonistic activity; Endophytic fungi; *Trichoderma dorotheae*; Zingiberaceae

Introduction

Trichoderma spp. is a microbial community of rhizospheric and endophytic fungi commonly known as potential biofungicides and plant-growth-promoting rhizofungi. Members of *Trichoderma* are ubiquitous in nature, explaining their broad colonization across plant species (Mukesh *et al*. 2016). So far, bio-prospective studies upon their use as biocontrol agents have been conducted especially against phytopathogenic fungi. Oil palm plantantions in North Sumatra harbor two endophytic fungal isolates, identified as *T. harzianum* and *T. viride*, reported as potential anti-*Ganoderma boninense* (Dharmaputra and Tjitrosomo 1990). Field studies showed that *T. harzianum* suppresses the growth of *Rigidoporus microporus*, a notable phytopathogen of the rubber tree (Jayasuriya and Thennakoon 2007). The biological agent, *T. harzianum*, isolate of Nigeria, was reported as a prominent biocontrol of *Fusarium oxysporum* based on colony growth inhibition in dual culture assay (Nwankiti and Gwa 2018). Discovering new strains of *Trichoderma* from prospective sources, such as internal environments of medicinal plants, may prove to be an effective strategy to reveal other potential strains. Endophytic micro-organisms are microbial associates of plants, with no specific role as pathogens, rather supporting the growth of plant species. Research conducted on these life habits has opened up ways of finding novel bioactive compounds in limited plant compound conditions (Kaul *et al*. 2012). In addition, plants with a hardy nature that tolerate many environmental stressors may also become a suitable source for isolation. Endophytic fungi are known to be better bioactive compound producers compared to bacteria, especially as antibacterial producers (Deshmukh *et al*. 2015). The synergy between bioactive compounds synthesized by plants may also contribute to the genetic elements of endophytic fungi and *vice versa*, leading to mutualistic interactions to sustain the life of both organisms (Ding *et al.* 2018). Some strategies in isolating endophytic micro-organisms, especially endophytic *Trichoderma*, have been reported, one of which is derived from medicinal plant

sources (Strobel 2003; Strobel and Daisy 2003). Numerous reports have revealed the occurrence of *Trichoderma* in plants with medicinal properties. *T. citrinoviride* strain PG87 isolated from *Panax ginseng* exhibited considerable mycoparasitism against ginseng pathogens (Park *et al*. 2018). A *Trichoderma* strain isolated from the medicinal plant family Cupressaceae produced significant antimicrobial metabolites. Two species, *T. atroviride* and *T. koningii*, are reported to synthesize some agrochemical compounds and drug materials with potential in biocontrol applications (Hosseyni-Moghaddam and Soltani 2014). The Zingiberaceous species are medicinal plants commonly cultivated in the tropics. Due to their high bioactive content in rhizome and other parts, these plant families have the potential to contain endophytic fungi (Habsah *et al*. 2000). In Indonesia, various parts of these plants have been used traditionally and locally as ethno-medicines (Auliani *et al*. 2014; Hartanto *et al*. 2014). Efforts have been made to explain the occurrence of endophytic fungi from the species. This study was conducted using 20 strains of *Trichoderma* representing five species of native Zingiberaceae: *Alpinia* spp., *Amomum centrocephalum*, *Elettaria* spp., *Etlingera elatior*, and *Hedychium coronarium.* Most strains were stronger antagonists to phytopathogenic fungi than against pathogenic bacteria. Molecular identifications were carried out to provide precise information of potential strains.

Materials and Methods

Plant materials

Samples of wild Zingiberaceae were collected during exploration in Sibayak Forest, located in Deli Serdang Regency, North Sumatra. Samplings were conducted incidentally without considering any climate and spatial factors. Plants anchored to soil were dug up and cut to separate its shoots and roots. The root or rhizome were wrapped with paper and stored in plastic bags. Duplicate samples were collected separately and identifiedin Herbarium Medanese, Universitas Sumatera Utara, Indonesia. The five collected Zingiberaceous species were identified as: *Alpinia* spp., *A. centrocephalum, Elettaria* spp., *E. elatior* and *H. coronarium.* Rhizomes were then cut into smaller segments and composites were made by pooling segments into one bulk sample for each species of Zingiberaceae. The samples were then used in isolation.

Isolation of endophytic *Trichoderma sp.*

The surface sterilization method method was used to isolate endophytic *Trichoderma* (Yurnaliza *et al*. 2014). Bulk samples from each Zingiberaceous species were cleansed under flowing water to remove soil debris. Rhizomes were blot-dried and subsequently dipped invarious sterilizing solutions: 75% EtOH (2 min), 5.3% NaOCl (5 min), 75% EtOH (30 s), and finally rinsed off using sterile distilled water to remove remaining solutions. Sterile rhizomes were cut by sterile surgical blade into smaller pieces (±1–2 cm) and placed on 0.1% (w/v) Chloramphenicol–PotatoDextrose Agar (Oxoid™). Plates were incubated under ambient conditions and monitored for any growing fungal colonies for 3 days. Each fungal isolate was determinedbased onits unique feature of *Trichoderma* morphologies and given tentative identities.

Molecular identification based on ITS region

Isolation of fungal DNA genome was as per the technical procedure prescribed by Wizard® Genomic DNA Purification Kit Protocol (Promega Corp., Madison, U.S.A.). Fungal mycelium (± 0.5 g) was picked and crushed using micropestle ina microtube. Mycelium was inserted into a microtube containing 600 μL of both SDS Tris-HCl buffer pH 8.0 and Phenol:Chloroform solution and centrifuged at 4°C,10,000 rpm for 45 min. The supernatant was discarded and the kit nucleus lysis (300 μL) and protein precipitation solution (100 μL) were added into pellets. The pellets were centrifuged at 13,000 rpmfor 3 min to obtain supernatant containing DNA. The supernatant was moved into a new tube containing 300 μL isopropanol following further centrifugation at 13,000 rpm for 5 min. Pellets containing DNA genome were dissolved and preserved in Tris-EDTA buffer (100 μL). Estimation of DNA quantity was donebased on its absorbance in spectrophotometer ($A_{260/280}$ and $A_{260/230}$). The fungal rDNA or ITS region was amplified using ITS-1 and ITS-4 primers (Manter and Vivanco 2007) with a reaction mixture (40 μL) containing nuclease-free water (12 μL), GoTaq DNA Polymerase solution (20 μL), ITS-1F primer (2 μL), ITS-4 primer (2 μL), DNA template (4 μL) in an Eppendorf tube. The PCR in thermal cycle was programmed in 35 cycles as follows: Pre-denaturation (95°C, 3 min), denaturation (95°C, 45 s), annealing (55°C, 45 s), elongation (72°C, 45 s), and final extension (72°C, 7 min). The confirmation of successful PCR running was visualized on 1% agarose gel electrophoresis under UV illuminations. The PCR amplicons were then transported to Macrogen, Inc. (Singapore) for DNA sequencing.

Bioinformatics study

The raw ITS-5.8S rDNA sequences were qualitatively assembled and trimmed for bioinformatic analyses, using BioEdit v. 7.2. Consensus sequences were aligned with other fungal sequences obtained from GenBank database. Sequence database with highly similar percentage run using BLASTn was downloaded and stored for phylogenetic constructions. Selected *Trichoderma* isolates were then subjected to further species confirmation by aligning the sequences using MUSCLE feature in MEGA6.0 (Edgar 2004; Tamura *et al*. 2013) based on resemblance. Phylogenetic inference from bioinformatic analyses was constructed into a tree using neighbor-joining method and maximum composite likelihood statistical method with

1000 bootstrapping replications (Felsenstein 1985; Saitou and Nei 1987).

Antagonism assay against pathogenic microbes and phytopathogenic fungi

The pathogenic microbial strains used in this study were: *Staphylococcus aureus* ATCC® 29213™, *Escherichia coli* ATCC® 25922™ and a clinical strain of *Candida albicans* from the Hospital of Universitas Sumatera Utara. Both *S. aureus* and *E. coli* were first grown in nutrient agar (NA), while *C. albicans* were grown in potato dextrose agar (PDA) prior to antagonism assay. Antagonism was assayed in a dual culture plate inoculated with fungal isolates and indicator micro-organisms (Balouiri *et al*. 2016). Bacterial and yeast suspensions were prepared by swabbing microbial colonies and dipping them into sterile physiological saline solutions (OD_{600} = 0.5). One mL of cell suspension was poured in a petri dish containing 15 mL molten PDA (45°C) medium, pre-added with either 1% (w/v) yeast extracts for bacterial growthor 1% bacto-peptone (w/v) for *C. albicans* growth. Three fungal plugs were placed on top of the agar medium pre-inoculated with microbial lawn in triplicates. Plates were incubated under ambient condition for 2 days. Clear zones around fungal colonies were measured using digital caliper in millimeters (mm) unit, which indicated antagonism against tested microbes. Each inhibition zone (IZ) was categorized based on previous study (Lutfia *et al.* 2019a). The phytopathogenic fungal species used in this study were: *F. oxysporum*, *G. boninense* and *R. lignosus*, provided by the Laboratory of Microbiology, Department of Biology, Universitas Sumatera Utara, Medan, Indonesia. Phytopathogens were grown in PDA medium to obtain fresh mycelial plugs. Mycelial plugs of phytopathogens were then planted three days in advance at the center of new PDA medium, followed by agar plugs of endophytic fungi in antagonism assay with three replicates. Plates were incubated for 7 days at room temperature. Colony Growth Inhibition (CGI) of phytopathogenic fungi was calculated by the following formula (Bivi *et al*. 2010):

$$CGI\ (\%) = \frac{R_1 - R_2}{R_1} x\ 100$$

Where CGI is percentage (%) of the colony growth inhibition. R_1 represents the diameter of phytopathogens colony growth in the absence of fungal antagonist, R_2 represents the diameter of phytopathogens from the antagonist direction. Each CGI result was again categorized into four arbitary levels of antagonism: Very Strong (++++) CGI>75%, Strong (+++) 75≥CGI>50%, Mild (++) 50≥CGI>25%, Weak (+) 25≥CGI> 0%, and None (–) CGI = 0%. Selection of potential antagonists was done based on their levels of antagonism and scored with a numerical value or Scoring Value (SV): Very Strong (4), Strong (3), Mild (2), Weak (1) and None (0).

Results

Endophytic fungi of Zingiberaceous species

Based on isolation results, a total of 20 tentative *Trichoderma* strains from five rhizomes of selected Zingiberaceous species naturally inhabiting Sibayak Forest, North Sumatra, were successfully recovered. Three isolates of endophytic *Trichoderma* were isolated from both *Alpinia* spp. (Al01–Al03) and *A. centrocephalum* (Am01–Am03), two isolates from *Elettaria* spp. (El01, El02), five isolates from *E. elatior* (Ee01–Ee05), and seven from *H. coronarium* (He01–He07) as the most populous species. Sibayak Forest itself is one of the tropical montane forestsin North Sumatra, previously reported with a collection of 23 Zingiberaceous species inhabiting the forest area (Siregar and Pasaribu 2008). During our exploration, two genera assemblages namely *Alpinia* and *Elettaria* were recently found and regarded as new reports from the North Sumatra region.

Molecular identification of endophytic fungi

All morphotypes of *Trichoderma* isolates were then identified based on their ITS-DNA similarity among databases. All isolates showed good identification results, as seen from their high percentage of identities, high query covers and low *E*-value (Table 1).

Antagonistic test of endophytic Fungi

Antagonism test results are presented in Table 2. In general, all *Trichoderma* strains showed prominent inhibitory activities against phytopathogenic fungi in varying level of antagonism, with the strongest activities against *G. boninense.* Only one species, *T. hamatum* did not display any antagonistic activity against pathogen. The majority of isolates failed to display significant inhibitory activities against pathogenic microbes, especially against *C. albicans.* Isolate *T. dorotheae* 02 derived from *E. elatior* displayed a broad anti-microbial activity against all tested pathogenic microbes, with the highest result against *S. aureus*. *T. viride* displayed the best antagonistic activity against *S. aureus,* but failed to display any reaction to *E. coli* and *C. albicans*.

Designation of potential *Trichoderma* strain

Scoring value (SV) based on our pre-determined arbitary level of antagonisms was used to differentiate potential *Trichoderma* species from others. The overall scoring can be seen in Fig. 1. The top five strains of considerable SV were: *T. dorotheae* 02, *T. koningii*, *T. viride*, *T. koningiopsis*, and *T. dorotheae* 01. Isolation of genomic ITS-DNA region revealed a satisfactory result of DNA amplification (Fig. 2). Further identification based on phylogenetic inferrence was constructed using maximum

Table 1: Identification of *Trichoderma* isolates based on their BLASTn results of GenBank database

Code Isolates	Identification results	Query cover	E value	Ident.	Accession
Al01	*Trichoderma koningii*	100%	0.0	100%	MH862585.1
Al02	*Trichoderma neokoningii*	100%	0.0	100	MH863076.1
Al03	*Trichoderma caribbaeum* 01	100%	0.0	99.61%	MH863051.1
Am01	*Trichoderma koningiopsis*	100%	0.0	100%	NR131281.1
Am02	*Trichoderma caribbaeum* 02	100%	0.0	99.81%	NR137302.1
Am03	*Trichoderma hamatum*	98%	0.0	99.84%	Z48816.1
El01	*Trichoderma viride*	96%	0.0	100%	DQ677655.1
El02	*Trichoderma afroharzianum*	100%	0.0	100%	NR137304.1
Et01	*Trichoderma rifaii*	100%	0.0	100%	NR137305.1
Et02	*Trichoderma dorotheae* 01	100%	0.0	100%	MH863050.1
Et03	*Trichoderma caribbaeum* 03	100%	0.0	100%	MH863051.1
Et04	*Trichoderma gamsii*	100%	0.0	100%	NR131317.1
Et05	*Trichoderma dorotheae* 02	99%	0.0	99.50%	MH863050.1
He01	*Trichoderma atroviride*	100%	0.0	100%	AF456917.1
He02	*Trichoderma brevicrassum*	100%	0.0	100%	NR154583.1
He03	*Trichoderma viridescens*	100%	0.0	99.81%	NR138429.1
He04	*Trichoderma lixii*	100%	0.0	99.68%	NR131264.1
He05	*Trichoderma longibrachiatum*	100%	0.0	100%	MH859229.1
He06	*Trichoderma dorotheae* 03	100%	0.0	100%	MH863050.1
He07	*Trichoderma hispanicum*	100%	0.0	100%	NR138451.1

Table 2: Result of antagonism assay shown by endophytic *Trichoderma* isolates

Species	Antagonism levels					
	S. aureus[a)]	*E. coli*[a)]	*C. albicans*[a)]	*F. oxysporum*[b)]	*G. boninense*[b)]	*R. lignosus*[b)]
T. koningii[1)]	+++	-	-	++++	++++	+++
T. neokoningii[1)]	++	++	-	++	++++	-
T. caribbaeum[1)]01	-	-	-	+++	++++	+++
T. koningiopsis[2)]	+++	-	-	+++	+++	+++
T. caribbaeum[2)]02	-	-	-	++	+++	+++
T. hamatum[2)]	-	-	-	+++	-	+
T. viride[3)]	++++	-	-	+++	+++	+++
T. afroharzianum[3)]	-	-	-	++++	+++	+++
T. rifaii[4)]	-	-	-	+++	++++	+++
T. dorotheae[4)]01	++	-	-	+++	+++	+++
T. caribbaeum[4)]03	-	-	-	+++	++++	+++
T. gamsii[4)]	-	-	-	+++	+++	+++
T. dorotheae[4)]02	+++	++	++	+++	+++	+++
T. atroviride[5)]	+++	-	-	+++	++++	++
T. brevicrassum[5)]	+	-	-	++	+++	++
T. viridescens[5)]	-	-	-	+++	+++	+++
T. lixii[5)]	-	-	-	++	++	++
T. longibrachiatum[5)]	+	-	-	+++	+++	+++
T. dorotheae[5)]03	-	-	-	+++	+++	+++
T. hispanicum[5)]	-	-	-	++++	+++	+++

a) Antagonism levels based on Inhibition Zones (IZ) (mm): Very Strong (++++) IZ > 30 mm, Strong (+++) 30 ≥ IZ > 20 mm, Mild (++) 20 ≥ IZ > 10 mm, Weak (+) 10 ≥ IZ > 0 mm, and None (–) IZ = 0 mm

b)Antagonism levels based on Colony Growth Inhibition (%): Very Strong (++++) CGI >75%, Strong (+++) 75 ≥ CGI > 50%, Mild (++) 50 ≥ CGI > 25 mm, Weak (+) 25 ≥ CGI > 0%, and None (–) CGI = 0%.

Source: [1)] *Alpinia* spp., [2)] *Amomum centrocephalum*, [3)] *Elettaria* spp., [4)] *Etlingera elatior*, [5)] *Hedychium coronarium*

likelihood method, revealing significant result of molecular identification based on ITS-DNA region (Fig. 3).

Discussion

Previous studies have investigated the diversity of culturable endophytic fungi associated with some Zingiberaceous species in tropical forests of North Sumatra, Indonesia, with the main prospects as anti-microbial producing agents and plant- growth- promoting fungi (Hartanto *et al.* 2019; Lutfia *et al.* 2019b, c, d; Munir and Lutfia 2019). Due to the increasing attention and limited information on our indigenous endophytic strains originating from the native medicinal plants, we seek the possibility on characterizing all the fungal species with an array of functional properties in agricultural and medical fields. In the current study, we focus on the twenty *Trichoderma* species as the dominant endophytic mycofauna occupying similar niches in Zingiberaceae of North Sumatra. The *Trichoderma* strains were mostly recovered from *H. coronarium* with seven isolates coded as: He01–He07. In another study, among 15 fungal isolates recovered from various plant parts of *H. coronarium* cultivated in Western Ghats, India, only one isolate was identified as *Trichoderma* (Uzma *et al.* 2016). A study also reported a very low relative isolation frequency (<1%) of *Trichoderma* strains from *A. siamense* (Bussaban

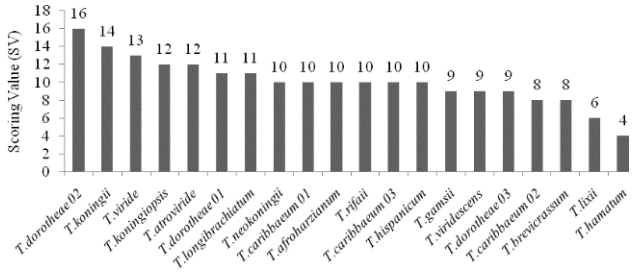

Fig. 1: Scoring of *Trichoderma* antagonistic results across species and hosts

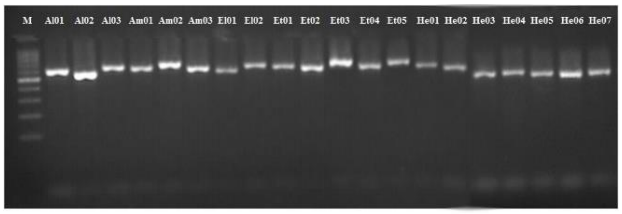

Fig. 2: Visualization of ITS-DNA amplicons with estimated product size ± 600 bp

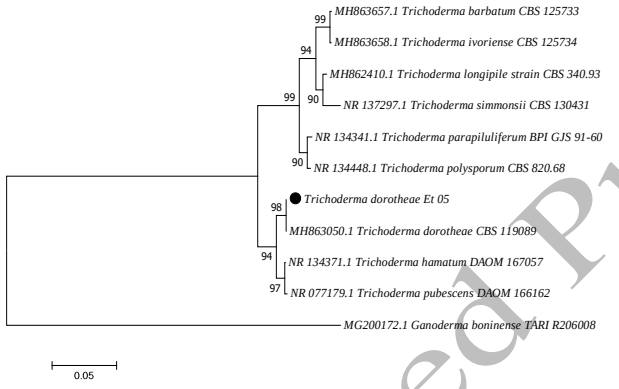

Fig. 3: Phylogenetic tree reconstruction of isolate Et05 (*T. dorotheae*) shown in black dots (•) among fungal databases. Inferred phylogenetic test were based on bootstrap test 1000x using Maximum Composite Likelihood method. All bootstrap value (BV) shown are ≥ 90% indicating significant results

et al. 2001). Although *Trichoderma* species is recognized as common mycoflora in the soil, it was revealed that there are habitat differences in both spatio-temporal aspects and host species, which caused the different numbers of culturable *Trichoderma* as endophytes (Gange *et al.* 2007). Meanwhile, two *Trichoderma* species were found colonized across plant hosts, namely *T. caribbeum* and *T. dorotheae*. Endophytic fungi have been reported to synthesize numerous antibacterial compounds, which promote their use as biocontrol agents of pathogenic bacteria (Deshmukh *et al.* 2015). However, their properties may not always be expressed due to varying testing methods. In this study, dual culture plate assay was used as a screening method for antagonisms of isolated *Trichoderma* species. The result showed a broad-spectrum of antagonistic activity by *T. dorotheae* 02 against all the pathogenic bacteria. The phenomenon may be due to the secretion of antibacterial metabolite into the pre-inoculated agar medium (plugs) in dual culture assay, leading to the diffusion of a more concentrated bioactive metabolites to the bacterial lawns. Hence, the isolates may be studied thoroughly for their potential in metabolite production as antibiotics. Previous investigation reported that the culture extract of *T. koningiopsis* QA-3 originating from *Artemisia argyi*, produced a strong antibacterial activity against *Escherichia coli*, while the compound was revealed as polyketides (Shi *et al.* 2017), while most of the isolates failed to display antagonistic activities against *C. albicans*, other approaches may be used by using their metabolite extracts directly for future assays. On the contrary, a rhizospheric *T. viride* isolated from a cucumber plantation, displayed a significant antifungal activity against *C. albicans* based on minimum inhibitory concentration (MIC) test of 100 *µ*g/disc by assessing their alcoholic-extract of fungal mycelia or biomass to the pathogenic yeast (Awad *et al.* 2018). *Trichoderma* spp. are mostly known to compete against surrounding microbes by producing extensive hyphal growth to outcompete their competitors within their niches and synthesizing other bioactive secondary metabolites (Verma *et al.* 2007). Several other biocontrol mechanisms promoted by these taxa are secretion of cell wall hydrolytic enzymes, niche exclusion through nutrients competition, mycoparasitism, and production of extracellular antibiotics (Mukesh *et al*. 2016). Based on the results using phytopathogenic fungi to assess inhibitory activities, *G. boninense* is the most sensitive strain tested in antagonistic assay. Two *Trichoderma* species, namely *T. harzianum* BIO-1, BIO-2 and *T. viride* isolated from oil palm rhizospheric habitat in North Sumatra, were reported to inhibit the colony growth of *G. boninense* with visual mechanism of hyphal lysis following a total overgrowth by antagonists (Dharmaputra and Tjitrosomo 1990). Rhizospheric *Trichoderma* was isolated from the soil of Kelantan, Malaysia. Among tested strains, *T. koningii* strain TK PB4 and PB2 displayed considerable inhibitory activities against *G. boninense*, as shown in plate assay (Naher *et al.* 2017). In another study also conducted in Malaysia, *T. harzianum* was reported as prominent antagonist against *G. Boninense*, using the dual culture method. The inhibition of the radial growth of the colony reached 72.06%, which is the highest percentage of recorded activity (Siddiquee *et al*. 2009). Other potential results were also shown by antagonistic *Trichoderma* against other two phytopathogenic fungi tested in this study, *F. oxysporum* and *R. lignosus*. *T. dorotheae* 02 isolated from *Etlingera* was considered to have the most potential *Trichoderma* isolate found in this study. The species has been reported as antagonistic fungi in previous reports. Isolate, *T. dorotheae* KUC1459 from woods in Korea revealed strong antagonistic effects towards wood-damaging

fungi: *Fomitopsis palustris*, *Gloeophyllum trabeum*, *Trametes versicolor*, *Ophiostoma floccosum*, *O. koreanum* and *O. piceapardum*. The isolate was characterized by its strong exo-chitinase activity, following a production of volatile antibiotics against tested phytopathogenic fungi (Lee *et al*. 2012). While this study only reflects the significance of the *Trichoderma* isolates owing to their potential properties as antifungi, future considerations must be evaluated specifically to characterize their metabolites and possible identification, since the same *Trichoderma* species or strains may differ in the production of bioactive metabolites.

Conclusion

Twenty *Trichoderma* strainswere successfully recovered, identified using 5.8-ITS rDNA molecular marker, and found to colonize rhizomes of five native Zingiberaceous species, namely: *Alpinia* spp., *A. centrocephalum*, *Elettaria* spp., *E. elatior*, and *H. coronarium*. The most commonly isolated *Trichoderma* species were *T. carribaeum* and *T. dorotheae*. Isolate *T. dorotheae* 02 from *Hedychium* was found to be the most potential antagonist against all the pathogenic bacteria and phytopathogenic fungi.

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Author contributions

AL conceived the original idea and performed the laboratory experimentation. EM interpreted the results and wrote the manuscript. Y analyzed the data and helped with the manuscript preparation.

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