Antifungal activity and IAA production by endophytic fungi isolated from *Elettaria* sp.

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**Abstract.** Medicinal plants may become a favourable habitat for associative microorganisms, especially endophytic microbes inhabiting Zingiberaceae species. Preliminary investigation has revealed an assemblage of endophytic fungi colonizing the healthy rhizome of *Elettaria* sp. collected from Sibayak Forest, North Sumatra based on molecular evidences. This study investigated the IAA-producing and antifungal activity from *Elettaria* endophytic fungi identified as *Curvularia lunata*, *Pholiota multicingulata*, *Trichoderma atroviride*, *T. harzianum* and *Schizophyllum commune*. Quantification of IAA was based on colorimetry method using Salkowsky reagent which produced pinkish to reddish solution indicating the presence of IAA. Antagonistic test was based on dual culture assay measured in colony growth inhibition (%). Statistical test to signify the differences were analysed using one-way ANOVA. *C. lunata* produced the highest IAA concentration of 45.17 µg.mL⁻¹ followed by *S. commune*, and *T. atroviride* with concentration of 11.7 and 5.27 µg.mL⁻¹, respectively. The strongest antagonistic activities were displayed by *T. harzianum* against both *G. boninense* (CGI>75%) and *R. lignosus* (CGI>50%). In addition, the strongest endophytic fungus against *F. oxysporum* was *P. multicingulata* (CGI>70%). Further investigations are being conducted to elaborate other plant growth promotion properties and even characterization of bioactive metabolites produced by *Elettaria* endophytic fungi.

1. Introduction

Zingiberaceae is an important medicinal plant family contributing to the composition and ethnobotany of traditional remedies in Indonesia [1]. Members of Zingiberaceae have been studied through laboratory test revealing many biological activities, such as anti-allergic, analgesic, anthelmintic, antimicrobials, antioxidants, and other physiological effects to health [2-5]. Currently, exploration of microbial associates, in specific to endophytic fungi has been carried out from medicinal plant sources. Medicinal plant may harbour beneficial endophytic fungi which play role in discovery of novel compounds with promising biological activities as its host [6-8].

Antibiosis is an example of properties of antagonistic microbial endophytes to support the growth of its host. Other beneficial properties which may be exposed by endophytic microorganisms, are their ability to produce exogenous phytohormone, e.g., auxin as plant growth promoting compound [9-11]. Endophytic fungi are able to synthesize various phytohormones, for example the indole-3-acetic acid (IAA) or auxin in laboratory scale as reported from previous studies.
Sixteen fungal isolates of South Sulawesi local aromatic rice have been reported to synthesize IAA in laboratory experiment [12]. Three endophytic fungal isolates from *Hottuyinia cordata* were reported to produce IAA and antagonists to *Rhizoctonia solani* [13]. Cultured endophytic fungi from *Elettaria* (Zingiberaceae) has been reported from previous study, revealing the occurrence of five endophytic fungal species, i.e., *C. lunata, P. multicingulata, T. atroviride, T. harzianum* and *S. commune* [14]. All isolated endophytic fungi were known as antagonists to test bacterial pathogens. However, the information about their plant growth promoting properties, especially IAA synthesis and antifungal activity are still unknown and needed to be investigated.

2. Materials and methods

2.1. Fungal isolates

A collection of endophytic fungi was used in this study, namely *C. lunata, P. multicingulata, T. atroviride, T. harzianum* and *S. commune* which were isolated from the rhizome part of *Elettaria* in Sibayak Forest, North Sumatra [14]. The fungal isolates were subcultured into new Potato Dextrose Agar (PDA) medium from stock cultures prior experimentation.

2.2. IAA production

The fungal isolates were subjected to batch fermentation of extracellular IAA. Two agar plugs were inoculated into 250-mL flask containing 100 mL of Czapek-Dox broth + L-tryptophan (0.1% w/v). The fermentation was done in ambient temperature for 8 days without agitation. After incubation, the mediums were filtered using Whatman filter paper No.1 to remove fungal biomass, following a centrifugation at 10,000×g for 10 min [15].

2.3. IAA quantification

Each cultural filtrates (1 mL) were mixed in 2mL Salkowsky indicator (150 mL H₂SO₄, 250 mL distilled water, 7.5 mL 0.5 M FeCl₃.H₂O) and incubated in the dark for 30 min. The formation of pink to red colour in solution indicates the presence of IAA while the formation of yellow colour indicates a low to negative yield of IAA [16]. The *in vitro* IAA production was compared to the standard IAA concentration (0–100 μg.mL⁻¹) by plotting the absorbance of sample (A₅₃₀) with the regression formula of pure IAA (Sigma, USA) in μg.mL⁻¹ of their absorbances. All experiments were done in triplicates.

2.4. Antagonistic assay

A collection of phytopathogenic fungi, namely *F. oxysporum, G. boninense* and *R. lignosus* were subcultured from agar slants into fresh PDA plate. Mycelial plugs from each phytopathogenic fungi were placed at the centre of PDA medium three days in advance, followed by the endophytic fungal plugs. Antagonistic test was performed in triplicates and incubated for 7 days at ambient temperature.

2.5. Data analysis

Data of IAA production were presented in mean ± S.D and were analysed using one-way ANOVA for statistical differences of *P* < 0.05. Colony Growth Inhibition (CGI) as antifungal activity from endophytic fungi was calculated using following formula [17]:

\[
CGI(\%) = \frac{(R_1 - R_2)}{R_1} \times 100\%
\]

Where, *R₁* represents the diameter of the colony of phytopathogenic fungi without endophytic fungi and *R₂* represents the diameter of the colony of phytopathogenic fungi towards the growth of endophytic fungi. Each CGI percentage was scored as follows: CGI > 75% or Very Strong (+++), 75 ≥ CGI > 50% or Strong (++), 50 ≥ CGI > 25 mm or Mild (+), 25 ≥ CGI > 0% or Weak (+), and CGI = 0% or None (−). Graphical images were generated from GraphPad Prism 8.0.2.
3. Results and discussion
The results showed that three endophytic fungal species, namely *C. lunata*, *S. commune*, and *T. atroviride* were able to produce extracellular IAA in different concentrations (figure 1). The culture filtrate was reacted with Salkowsky reagent, producing pinkish or reddish colour which indicated presence of IAA compound (figure 2). Meanwhile, two isolates, namely *P. multicingulata* and *T. harzianum* were not able to produce exogenous IAA into fermentation broth. Isolate *C. lunata* produced the highest IAA concentration reaching of 45.17 µg.mL⁻¹ followed with *S. commune* and *T. atroviride* with concentration of 11.7 and 5.27 µg.mL⁻¹, respectively.

Endophytic fungus, *C. lunata* showed the highest IAA production which is considerably higher than previous reports. Endophytic *L. pseudotheobromae* isolated from the rubber tree (*Hevea brasiliensis*) was reported to produce 37.034 µg.mL⁻¹ IAA in Czapek-Dox broth under agitation for 7 days [13]. Endophytic *F. oxysporum* isolated from Indian ginseng, *Withania somnifera* produced 31.6 µg.mL⁻¹ of exogenous IAA into Czapek-Dox broth [15]. In similar plant, *Aspergillus awamori* strain w11 produced 24.8 µg.mL⁻¹ IAA which successfully improves the growth of *Zea mays* [18]. Although
C. lunata is mostly known as phytopathogenic fungi, application of its culture filtrate containing IAA may be potent in future study to promote plant growth considering the safety issue.

Other endophytic fungus, S. commune is also able to produce IAA in which already reported from previous study, but not as endophytic associates from Zingiberaceae [19]. An unusual result was obtained from T. atroviride and T. harzianum in our study, which produced no IAA if compared to previous studies [20,21]. IAA biosynthesis is convergently evolved in various organisms, from bacteria, fungi to plants which shows that IAA may act as a widespread physiological code in promoting symbiosis. Furthermore, study on endophytic fungi from Zingiberaceous species as plant growth promoters is still limited, in contrary to its endophytic bacteria. Endophytic bacteria inhabiting rhizome of Zingiber officinale was reported to produce IAA qualitatively and quantitatively and promoted the growth of selected plant [22-25].

![Figure 3. Antifungal activity of endophytic fungi expressed into Colony Growth Inhibition (CGI, %) against phytopathogenic fungi.](image)

Meanwhile, antifungal activity from each endophytic fungi was tested against phytopathogenic fungi during 7 days of incubation in dual culture. Results are presented in figure 2. The antifungal activities of each endophytic fungi are different according to phytopathogenic fungi tested. The strongest activity obtained in this study was from T. harzianum against both G. boninense and R. lignosus while no antifungal activity observed from P. multicingulata against R. lignosus. The most potential isolate against F. oxysporum was P. multicingulata with CGI percentage of 72.75%.

Trichoderma harzianum has been reported from previous study as potential soil-borne fungal antagonist to various phytopathogenic fungi, through mechanism or competitive exclusion or antibiosis as unique feature of all members of Trichoderma [26]. Soil-borne T. harzianum from Malaysian oil palm plantation was isolated and studied for its antifungal activity against G. boninense. The study reported that T. harzianum strain PER 71 inhibited the radial growth of G. boninense colony with percentage reaching 72.06%. In addition, endophytic Trichoderma spp. isolated from oil palm midrib showed significant inhibitions to G. boninense with the highest CGI percentage of 80% [27]. Meanwhile, no report on P. multicingulata as antagonist to F. oxysporum or to other phytopathogenic fungi which indicate a new report upon this finding.

In general, very little was known on endophytic microbes from Elettaria. Owing to this fact, further investigations are needed to elaborate other plant growth promoting properties by these fungi or even metabolite extraction with antimicrobial properties from other Zingiberaceous species.
4. Conclusions

Three endophytic fungi, namely Curvularia lunata, Schizophyllum commune, and Trichoderma atroviride isolated from rhizome of Elettaria in Sibayak Forest, North Sumatra, are able to produce IAA in fermentation broth with concentration of 45.17, 11.7 and 5.27 µg.mL\(^{-1}\) respectively. All endophytic fungal isolates produced antifungal activity at least to one phytopathogenic fungi tested. The strongest endophytic fungus was T. harzianum against G. boninense and R. lignosus based on CGI percentage.

References

[1] Fernando O, Nursyahra and Rizki 2008 Studi pemanfaatan tumbuhan familia zingiberaceae yang berasal dari kecamatan Luhak Nan Duo kabupaten Pasaman Barat [Study of the utilization of zingiberaceae family plants with medicinal properties in Luhak Nan Duo sub-district, West Pasaman district] Pendidik. Biol. 4 pp 1–5

[2] Sabulal B, George V, Dan M and Pradeep N S 2007 Chemical Composition and Antimicrobial Activities of the Essential Oils from the Rhizomes of Four Hedychium Species from South India J. Essent. Oil Res. 19 pp 93–7

[3] Kumar R, Prakash O, Pant A K, Isidorov V A and Mathela C S 2012 Chemical composition, antioxidant and myorelaxant activity of essential oils of Globba sessiliflora Sims J. Essent. Oil Res. 24 pp 385–91

[4] Juwita T, Melyani Puspitasari I and Levita J 2018 Torch Ginger (<I>Etlingera elatior</I>): A Review on its Botanical Aspects, Phytoconstituents and Pharmacological Activities Pakistan J. Biol. Sci. PJBS 21 pp 151–65

[5] Chan E W C and Wong S K 2015 Phytochemistry and pharmacology of ornamental gingers, Hedychium coronarium and Alpinia purpurata: a review J. Integr. Med. 13 pp 368–79

[6] Strobel G A 2003 Endophytes as sources of bioactive products Microbes Infect. 5 pp 535–44

[7] Strobel G 2018 The Emergence of Endophytic Microbes and Their Biological Promise J. Fungi 4 57

[8] Aly A H, Debbab A and Proksch P 2011 Fungal endophytes: Unique plant inhabitants with great promises Appl. Microbiol. Biotechnol. 90 pp 1829–45

[9] Verma M, Brar S K, Tyagi R D, Surampalli R Y and Valéro J R 2007 Antagonistic fungi, Trichoderma spp.: Panoply of biological control Biochem. Eng. J. 37 pp 1–20

[10] Lutfia A, Munir E and Yurnaliza 2019 Antagonistic Endophytic Fungi of Globba pendula Roxb. from Taman Hutan Raya, North Sumatra against Staphylococcus aureus ATCC® 29213 \(^{\text{TM}}\)IOP Conf. Ser. Earth Environ. Sci. 305 012003

[11] Lutfia A, Munir E and Yurnaliza 2019 Antagonistic Endophytic Fungi of Hedychium coronarium Roxb. from Sibayak and Taman Hutan Raya, North Sumatra against Staphylococcus aureus ATCC® 29213 TM IOP Conf. Ser. Earth Environ. Sci. 305 012002

[12] Syamsia, Kuswinanti T, Syam’un E and Masniawati A 2015 The Potency of Endophytic Fungal Isolates Collected from Local Aromatic Rice as Indole Acetic Acid (IAA) Producer Procedia Food Sci. 3 pp 96–103

[13] Aramsirirujwet Y, Gumlangmak C and Kitpreechavanich V 2016 Studies on Antagonistic Effect Against Plant Pathogenic Fungi from Endophytic Fungi Isolated from Hottuynia cordata Thunb. and Screening for Siderophore and KLU Res. J. 21 pp 55–66

[14] Munir E, Lutfia A and Yurnaliza 2019 Records of Culturable Endophytic Fungi Inhabiting Rhizome of Elettaria in Hutan Sibayak, North Sumatera IOP Conf. Ser. Earth Environ. Sci. 305 012004

[15] Mehmood A, Khan N, Irshad M, Hamayun M, Husna I, Javed A and Hussain A 2018 IAA Producing Endophytic Fungus Fusarium oxysporum w/Colonize Maize Roots and Promoted Maize Growth Under Hydroponic Condition Eur. J. Exp. Biol. 08 pp 1–7

[16] Meudt W J and Gaines T P 1967 Studies on the oxidation of Indole-3-acetic acid by peroxidase enzymes. I. colorimetric determination of Indole-3-acetic acid oxidation products Plant Physiol.
[17] Bivi M R, Farhana M S N, Khairulmazmi A and Idris A 2010 Control of Ganoderma boninense: A Causal Agent of Basal Stem Rot Disease in Oil Palm with Endophyte Bacteria In Vitro Control of Ganoderma boninense: A Causal Agent of Basal Stem Rot Disease in Oil Palm with Endophyte Bacteria In Vitro Int. Journa Agric. Biol. 12 12 pp 833-9

[18] Mehmood A, Hussain A, Irshad M, Hamayun M, Iqbal A and Khan N 2019 In vitro production of IAA by endophytic fungus Aspergillus awamori and its growth promoting activities in Zea mays Symbiosis 77 pp 225–35

[19] Epstein E and Miles P G 1967 Identification of Indole-3-Acetic Acid in the Basidiomycete Schizophyllum commune Plant Physiol. 42 pp 911–4

[20] Saber W I A, Ghoneem K M, Rashad Y M and Al-Askar A A 2017 Trichoderma Harzianum WKY1: an indole acetic acid producer for growth improvement and anthracnose disease control in sorghum Biocontrol Sci. Technol. 27 pp 654–76

[21] Gravel V, Antoun H and Tweddell R J 2007 Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: Possible role of indole acetic acid (IAA) Soil Biol. Biochem. 39 pp 1968–77

[22] Rohini S, Aswani R, Kannan M, Sylas V P and Radhakrishnan E K 2018 Culturable Endophytic Bacteria of Ginger Rhizome and their Remarkable Multi-trait Plant Growth-Promoting Features Curr. Microbiol. 75 pp 505–11

[23] Jasim B, Joseph A A, John C J, Mathew J and Radhakrishnan E K 2014 Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of Zingiber officinale 3 Biotech 4 pp 197–204

[24] Kartikawati A and Gusmaini 2018 The Potency of Endophytic Bacteria Isolated From Red Ginger to Enhance Black Pepper Seedlings growth Bul. Penelit. Tanam. Rempah dan Obat 29 pp 37–46

[25] Chen T, Chen Z, Ma G H, Du B H, Shen B, Ding Y Q and Xu K 2014 Diversity and potential application of endophytic bacteria in ginger Genet. Mol. Res. 13 pp 4918–31

[26] Mukesh S, Vipul K, Mohammad S, Sonika P, ey and Anuradha S 2016 Trichoderma- a potential and effective bio fungicide and alternative source against notable phytopathogens: A review African J. Agric. Res. 11 pp 310–6

[27] Yurnaliza, Aryantha I N P, Esyanti R R and Susanto A 2014 Antagonistic Activity Assessment of Fungal Endophytes from Oil Palm Tissues Against Ganoderma boninense Pat Plant Pathol. J.13 pp 257–67

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