Screening of antagonistic fungi from *Etlingera littoralis* (J.König) Giseke rhizome in Sibolangit Forest, North Sumatra

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**Abstract.** *Etlingera littoralis* (J.König) Giseke is a medicinal plant species from Zingiberaceae in North Sumatra, Indonesia. The species has close relationship to *Etlingera elatior* with lesser known potential and information on its microbial associates. Endophytic fungi were screened and explored through various species of Zingiberaceae with the aim of finding antagonistic fungi against pathogenic bacteria. Isolation of endophytic fungi was based on surface sterilization and direct plating of rhizome part into isolation medium. Endophytic fungal isolates were characterized based on its morphological characteristics. Antagonism assay was employed against representative pathogenic bacteria: *Staphylococcus aureus* (ATCC® 29213™), Methicillin-resistant *Staphylococcus aureus* (ATCC® 43300™), *Escherichia coli* (ATCC® 25922™), and Enteropathogenic *E. coli* K11. We obtained six endophytic fungal isolates which were antagonists to at least one of tested pathogen. Majority of isolates produced antagonistic activities against *S. aureus* while only one isolate, namely Eel05SU was known to inhibit all tested pathogenic bacteria. Identification of potential isolate is currently carried out to gain information of its identity along with deeper investigations of its biological properties.

1. **Introduction**

Bioprospection study of endophytic fungi from medicinal plant source has occurred in increased rate recently [1]. The fact that healthy and asymptomatic plant may harbor vast number of microorganisms have drawn the attention to many microbiologist in studying their potential. Endophytic fungi, by far hold the pre-dominance over bacteria regarding the synthesis of novel compounds [2]. The probability on finding fungal strains with desirable traits are now conducted by exploring and studying plant species with prospective biological properties in the field of new drugs development [3]. Medicinal plants are thought to be a valuable source of endophytic fungi [4].

Assessment of endophytic fungi with potential inhibitory activity against pathogenic bacteria have been explored from notable medicinal plant, Zingiberaceae in Indonesia [5–7]. *Hedychium coronarium* isolated from North Sumatran tropical forest was reported to harbor 28 endophytic fungi [8]. Other known Zingiberacean species, *Globba pendula* was also reported to harbor 16 endophytic fungi in similar study [9]. Bioprospection study of endophytic fungi inhabiting *Anomum centrocephalum* has revealed a number of endophytic fungal species with antagonistic activity against clinical pathogenic bacteria and phytopathogenic fungi [10]. Through molecular identification, it has...
been reported that several endophytic fungal species may be originated from soil microorganisms [11]. Efforts to reveal other symbiotic fungi from less-studied Zingiberacean species are still continuing.

Torch ginger, *Etlingera elatior* is known as a member of Zingiberaceae with prospective pharmacological and biological properties exerted from its natural compounds [12]. Distribution of *Etlingera* spp. is abundant in tropical region, especially Southeast Asia [13]. However, not many species have been studied, leaving the trace of possibility on finding different species of endophytic fungal species. In addition, study of endophytic fungi from *Etlingera littoralis* as member of Zingiberaceae is still limited and less studied. In this study, we evaluated the antagonistic activity of endophytic fungal isolates against representative bacterial pathogens from reference to resistant strains. We found a promising result in promoting further investigations on these isolates in the future.

2. Methodology

2.1. Plant material
Fresh rhizome of *Etlingera littoralis* (J.König) Giseke was sampled from forest area of Sibolangit Forest, Deli Serdang regency, North Sumatra, Indonesia. The rhizome part was identified tentatively by observing distinctive floral parts. The rhizome was dug and cut below ground level. Samples were placed in sterile ziploc bags, maintained at 4°C in an ice box during transport and processed within 48 hr. Whole specimens were taken and authenticated by local Herbarium Medanese, Department of Biology, Universitas Sumatera Utara, Medan, Indonesia.

2.2. Isolation of endophytic fungi
Isolation of endophytic fungi from rhizome part were based on surface sterilization principle [14]. The rhizome samples were rinsed using tap water for 20 min, and cut into smaller fragments (1–2 cm). The fragments were surface-sterilized by immersing into sterilizing solutions: 75% ethanol (2 min), 5.3% NaOCl (5 min), and 75% ethanol (30 secs). The fragments were rinsed again with sterile distilled water to remove disinfectants. Fragments were dried using Whatman filter paper No.1 following cut into smaller pieces. The pieces were plated on Potato Dextrose Agar medium (Oxoid™) supplemented with antibiotic, chloramphenicol. Plates were incubated in ambient condition for 3 days. Fungal mycelium growing from each pieces was then purified. Fungal isolates were morphologically differentiated.

2.3. Antagonism assay
Two gram positive bacteria: *Staphylococcus aureus* (ATCC® 29213™) and Methicilin-resistant *Staphylococcus aureus* (ATCC® 43300™), and two gram negative bacteria: *Escherichia coli* (ATCC® 25922™), and Enteropathogenic *E. coli* K11 were obtained from the Hospital of Universitas Sumatera Utara, Medan, Indonesia. Procedure on testing antagonistic activity of endophytic fungi was based on agar plug diffusion assay [15]. Each pathogenic strains were prepared as inoculum by swabbing colonies into 0.95% NaCl solution to obtain optical density or OD600 of 0.5. One mL of cell suspension was inoculated into 20 mL of molten PDA (45°C), supplemented with 1% (w/v) yeast extracts for bacterial growth. The molten PDA was then poured to obtain microbial lawns. Three plugs of fungal isolates were placed on top of PDA in triplicates. The medium was incubated for 2 days in ambient temperature. Fungal isolates producing any clear zones indicate inhibitory activities or antagonisms which were measured using standard caliper in mm.

3. Results and Discussion
During our exploration in Sibolangit Forest, we attempted to collect other Zingiberacean species along with the sample, *Etlingera littoralis* (Figure 1). The species is identified based on its floral parts which be later confirmed through herbarium checking. Fresh rhizomes and its leaves were aromatic in scent as signature of Zingiberacean species. Previous study have reported similarities among essential oil
between *E. elatior* and *E. littoralis* indicating some chemotaxonomic marker to differentiate between species [16].

Isolation of endophytic fungi resulted in six fungal morphotypes which were characterized based on colony morphologies (Table 1). We found that most endophytic fungi were circular in colony appearance with different coloration of mycelium or conidia between surface and bottom of colonies. Hence, all isolates were subjected to antagonism assay against pathogenic bacteria. The results are presented in Figure 2.

Table 1. Morphological characteristics of endophytic fungal isolates of *Etlingera littoralis* rhizome

| Code   | Form   | Elevation | Margin     | Color Surface     | Color Bottom    | Texture   | Concentric ring | Radial line |
|--------|--------|-----------|------------|-------------------|-----------------|-----------|-----------------|-------------|
| Eel01SU| Irregular| Flat      | Undulate   | Grey              | Black           | Dull      | -               | -           |
| Eel02SU| Circular| Flat      | Filiform   | White             | White           | Flourish  | -               | -           |
| Eel03SU| Circular| Flat      | Filiform   | Light brown       | Light brown     | Cottony   | -               | -           |
| Eel04SU| Circular| Umbonate  | Entire     | Purple            | Cream           | Smooth    | -               | -           |
| Eel05SU| Circular| Flat      | Entire     | Dark green        | Light green     | Flourish  | +               | -           |
| Code    | Form   | Elevation | Margin  | Color Surface | Color Bottom | Texture | Concentric ring | Radial line |
|---------|--------|-----------|---------|---------------|--------------|---------|-----------------|-------------|
| Eel06SU | Irregular | Raised  | Filiform | Dark green    | Light green  | Flourish | -               | -           |

Figure 2. Antagonism of endophytic fungi isolated from *Etlingera littoralis* rhizome

The results of antagonism assay showed that *S. aureus* ATCC® 29213™ was the most sensitive strain, followed with *Escherichia coli* ATCC® 25922™, EPEC and MRSA. In our previous studies, *S. aureus* was also reported as the most sensitive strain in preliminary screening of antagonistic strains [8,9]. Among isolates, we only obtained one isolate namely Eel05SU which exhibit antagonistic activity to all tested pathogenic bacteria. Occurrence of endophytic fungi originating from medicinal plants have been reported previously with biological prospects. Endophytic *Trichoderma citrinoviride* from *Panax ginseng* in China was reported to possess biocontrol activity against ginseng pathogens [17]. *Trichoderma koningiopsis* QA-3 isolated from the medicinal plant *Artemisia annua* in China was found to produce antibacterial activity against *E. coli* and phytopathogenic fungi through antibacterial test of its polyketides [18]. Endophytic fungus, *Penicillium frequetans* isolated from *Pinus roxburghii* in India was reported to *S. aureus*, *E. coli*, *Salmonella typhimurium*, *Candida albicans* and *Rhizoctonia solani* in dual culture assay [19]. Our isolate, Eel05SU isolated from *E. littoralis* then may reflect the possibility of finding new mechanism or compounds to combat infection by these pathogenic strains.

4. Conclusion
Six endophytic fungal species have been isolated from healthy *Etlingera littoralis* sampled from Sibolangit Forest, North Sumatra, Indonesia. The six isolates displayed distinctive morphologies and regarded as different species. Majority of fungal isolates were tested against pathogenic bacteria which resulted in one isolate namely Eel05SU with potential antibacterial activities against *Staphylococcus aureus* (ATCC® 29213™), Methicillin-resistant *Staphylococcus aureus* (ATCC® 43300™), *Escherichia coli* (ATCC® 25922™), and Enteropathogenic *E. coli* K11.
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