Isolation of endophytic fungi from *Styrax sumatrana* tree from Humbang Hasundutan, North Sumatra

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Abstract. Endophytic fungi are a group of microorganisms colonizing the plant tissue without exposing hazardous effects to their host. Higher plants have endophytic microbes that produce bioactive compounds or secondary metabolites. The aim of the study was to isolate and identify endophytic fungi from the leaves, stems and bark of *Styrax sumatrana*. Samples of *Styrax sumatrana* leaves, stems and bark were collected from Huta Gurgur Village, Dolok Sanggul District, Humbang Hasundutan Regency, North Sumatra. Potato dextrose agar (PDA) was used as an isolation medium for endophytic fungi. The collection of endophytic fungal isolates was identified molecularly using ITS1 and ITS4 primers. This study obtained a total of 12 isolates of endophytic fungi, in which five isolates from bark, five isolates from stems and two isolates from leaves. Based on molecular identifications, the 12 isolates belonging to four genera, namely *Fusarium*, *Phyllosticta*, *Neopestalotiopsis* and *Pithomyces*.

1. Introduction
Fungi are a group of eukaryotic microorganisms that have an important role in nature, agriculture, biotechnology and various living organisms. Fungi can be isolated from various ecosystems as well as terrestrial plant and marine plant tissue. Endophytic fungi are fungal associates inside of higher plant tissues that do not harm their hosts [1-4]. Endophytic fungi may protect its host plants from various environmental pressures [5,6] and help to promote their growth [7]. Various endophytic fungi possess a unique metabolic pathway which can synthesize the similar metabolites produced by their hosts [8] and also produce other biologically active compounds [9]. Endophytic fungi are recognized as the recent valuable sources of bioactive compounds or as alternative sources of phytocompounds produced by most plants [10]. Various compounds have been reported from endophytic fungal studies producing metabolites with bioactivities such as anticancer, antibiotics, antifungal, antiviral, insecticides, and phytohormones [11,12].

Each higher plant can be colonized by one or more endophytes. Endophytes have the ability to colonize plant tissue such as healthy foliar parts, stems, bark, roots, fruit, flowers and seeds of the host plant [13]. One interest of our native plant species to study is *Styrax*. Styrax plant produces benzoin rosin which is used in medicinal, ritual, pharmaceutical and has economic value. *Styrax sumatrana* is an endemic rosin producing plant from North Sumatra. The distribution of this plant is very limited so it needs to be conserved [14].
The presence of endophytes fungi may support this plant not to be harvested directly to obtain its bioactive compounds. So it is necessary to do exploration to obtain the collection of endophytic fungi with future perspectives of finding similar metabolites as *S. sumatrana*. The aim of this research was to isolate and identify the endophytic fungi from bark, leaves, and stems of *S. sumatrana* tree.

2. Materials and methods

2.1. Plant collection

*Styrax sumatrana* were randomly collected from Huta Gurgur Village, Dolok Sanggul District, Humbang Hasundutan Regency, North Sumatra. The plant parts collected were leaves, stems and bark. Plants were put in a plastic bag, then it has taken to the laboratory for endophytic fungi to be isolated.

2.2. Isolation of endophytic fungi

Barks, leaves, and stems were cleaned under running tap water, then surface-sterilized by soaking the plant parts into 70% EtOH (2 min), NaOCl (2 min), sterile distilled water (3×), and blot-dried [15]. After that, the dried parts were cut and plated on potato dextrose agar (PDA). Chloramphenicol (100 mg/L) was supplemented as bacterial antibiotics. The last rinse water from each part of the plant was used as a control. If after 7 days it is not contaminated, then the fungi that grow in the medium are endophytic fungi. Petri dish with cut plant parts was incubated for 7 days at 25°C. Colony growth was observed every day. Different colonies of fungi were purified and stored for identification.

2.3. Molecular identification of endophytic fungi

The identity of each fungal isolate was revealed by performing common procedures of DNA isolation, polymerase chain reaction (PCR), and bioinformatics study [16]. Isolation of fungal genomic DNA followed the technical procedure given by DNeasy Plant Mini kit, Amplification of fungal 5.8S rDNA was performed on ITS-rDNA region using ITS1 and ITS4 primers with a reaction mixture (25 µL) containing of Go Taq master mix solution (12.5 µL), DNA template (2 µL), each primer (1 µL), and nuclease-free water (8.5 µL). PCR reaction was specified as: 94°C (5 min), 94 °C (30 sec), 52 °C (30 sec), 72 °C (30 sec), and 72 °C (7 min) with 35 cycles. Confirmation of successful DNA amplification was visually determined on agarose gel electrophoresis (1.5%, w/v) and sent to First Base (Malaysia) for sequencing, followed by the sequence trimming, comparison, and database search using BLASTn feature from GenBank.

3. Results and discussion

3.1. Isolation of endophytic fungi

Of the six *S. sumatrana* trees that were sampled for their leaves, stems and bark, 12 isolates of endophytic fungi were successfully isolated and purified. Five isolates were isolated from the stems, five isolates from the bark and two isolates isolated from the leaves. Plants dynamically respond to a large number of microorganismal communities where some of these microbes enter and live inside their host’s plant tissue with no hazardous effect. Such microbial associations are called endophytes. Endophytes can be found in various types of plants [17]. The assemblage of endophytic fungi may be spatio-temporarily affected by the sampling site, time of sampling, environmental conditions, and seasons [18,19]. The existence of endophytic fungi in plants can increase plant resistance to disease and environmental stress so that it can increase plant growth and produce bioactive compounds [20]. Endophytic fungi were also reported to secrete indole acetic acid (IAA) and it can dissolve inorganic phosphate in soils to promote plant growth [21].
3.2. Molecular identification of endophytic fungi

The result performed with BLAST search showed that 12 isolates of endophytic fungi were identified as *Fusarium* sp, *Fusarium solani*, *Phyllosticta capitalensis*, *Neopestalotiopsis clavispora*, *Pithomyces chartarum*, and *Neopestatiopsis foedans* (table 1).

| Isolate Code | Species identified           | Similarity (%) | Origin of the isolate |
|--------------|------------------------------|----------------|-----------------------|
| DSST1B       | *Fusarium solani*            | 100            | √                     |
| DSST1L       | *Phyllosticta capitalensis*  | 99             |                      |
| DSST2B       | *Fusarium solani*            | 100            | √                     |
| DSST2W       | *Neopestalotiopsis clavispora* | 100         | √                     |
| DSST2L       | *Pithomyces chartarum*       | 100            | √                     |
| DSST3B1      | *Fusarium sp.*               | 99             | √                     |
| DSSTB2       | *Fusarium sp.*               | 99             | √                     |
| DSST5B1      | *Neopestalotiopsis foedans*  | 100            | √                     |
| DSST6W1      | *Fusarium solani*            | 100            | √                     |
| DSST6W2      | *Fusarium solani*            | 100            | √                     |
| DSST6W4      | *Fusarium sp.*               | 99             | √                     |
| DSST7W3      | *Fusarium sp.*               | 99             | √                     |

The biological diversity of endophytic fungi is abundant, especially both in temperate and tropical rain forests. The existence of endophytic fungi has been traced in nearly 300,000 plant species, where each plant may be colonized by one or more fungal species [22]. ITS1 and ITS4 primers are commonly used to identify endophytic fungi. The sequencing results from PCR are then compared with the available sequences from the GenBank [23]. All isolates were identified belonging to the Ascomycota group which was also similar to previous studies [24,25].

The species of endophytic fungi isolated from *S. sumatrana* tree varied among plant parts. There were 8 species of *Fusarium*, 2 species of *Neopestalotiopsis*, 1 species of *Phyllosticta* and 1 species of *Pithomyces*. Several species of *Fusarium* are known to produce bioactive compounds such as antitumor by *F. oxysporium*, antioxidant by *F. solani*, immunosuppressive by *F. subglutinans*, antimicrobial by *F. moniliforme*, with the majority of compounds identified as alkaloids [26]. Apart from being known as endophytes, the genus *Fusarium* also causes disease in plants. *Fusarium* causes root and wilt disease of various types in plants. The dominance of *Fusarium* was also documented as being isolated from the stem of *Azadirachta indica* with their ability in producing similar bioactive compounds as its host [26].

4. Conclusions

The total of 12 endophytic fungi isolates were isolated from *S. sumatrana* leaves, stems and bark. Based on molecular identification the twelve isolates included 4 genera namely *Fusarium*, *Phyllosticta*, *Neopestalotiopsis* and *Pithomyces*.

References

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Acknowledgements
This research was financially supported by Ministry of Research and Technology/National Research and Innovation Agency, Republic of Indonesia Number: 11/AMD/E1/KP.PTNBH/2020 dated May 11, 2020.