ABSTRACTS (MASTER THESIS)

Molecular Phylogenetic Analyses of Fungal Diversity in Agarwood from *Aquilaria malacensis*

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Introduction

Agarwood (known as Jinko in Japan), a dark resinous wood found in trees in Southeast Asia such as *Aquilaria* sp., has been traditionally used for incense and perfumes, and thus it has been considered a high-value non-timber forest product. Since agarwood is a biological product of the defensive reaction against fungal infection, a human-inducible stimulus that mimics the natural fungal infection phenomenon in trees is necessary for the industrial production of agarwood. The artificial formation of agarwood by forcible infection using a specific fungus may be a useful option for the industrial production of this wood. For this purpose, identification of the fungus responsible for agarwood formation is needed, but the information available on isolating this fungus is limited. In the present study, the fungal community existing in agarwood of *Aquilaria* sp. was investigated by polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE) and was compared with that in healthy wood of *Aquilaria* sp. to identify the fungus that causes agarwood formation.

Materials and methods

Eight wounded *Aquilaria malacensis* trees (T1-T8) were selected from two different islands in Indonesia (Sumatra and Kalimantan Island). Genomic DNAs were extracted from 90 mg of milled wood samples using the DNeasy Plant Mini Kit (Qiagen). PCR was done in a reaction volume of 50 µl containing 2 mM of dNTPs, 25 mM $MgSO_4$, 10 x PCR buffer for KOD ver. 2, 1 U of KOD plus ver. 2 DNA polymerase, 10 µM of primers (ITS1-F and ITS4), and the extracted genomic DNA already mentioned. After the reaction, PCR products were analyzed by electrophoresis on 1% agarose gel and then underwent DGGE analysis using 8% polyacrylamide gel containing a 20-70% concentration gradient of denaturant. The separated DNA fragments were subjected to sequencing analysis. The determined nucleotide sequences were used for a BLAST search using the blastn algorithm at the NCBI website.

Results and discussion

When the PCR products were subjected to DGGE analysis, a total of 124 DNA bands were separated based on their different levels of mobility on the gel for the samples from Kalimantan. Among these DNA bands, the nucleotide sequences of 56 DNA fragments (37 fragments from dark wood and 19 fragments from white wood) were identical with those from filamentous fungi. Under conditions similar to those for the Kalimantan samples, the Sumatra samples were subjected to DGGE analysis; 85 of the DNA bands were separated, and among them 40 of the DNA fragments including 29 fragments from dark wood and 11 DNA fragments from white wood were also identical with those from filamentous fungi. In this study, Ascomycetes and Basidiomycetes were discovered in both areas. This study shows the advantages of the DGGE technique. PCR-DGGE obtained overall data on the fungi species that exist in natural agarwood. Other studies reported that several fungi were isolated from agarwood. In the present study, we identified more fungal species, suggesting that agarwood might be formed by the association of multiple fungi.

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