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SCREENING AND IDENTIFICATION OF FUNGI ASSOCIATED WITH NATURAL AND ARTIFICIALLY INDUCED AGARWOOD FROM *Aquilaria malaccensis*

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ABSTRAK

Aquilaria malaccensis ialah sejenis pokok tropika yang mengeluarkan resin teras yang berharga iaitu gaharu menerusi proses semulajadi daripada kecederaan fizikal secara semulajadi atau buatan atau jangkitan microorganisma. Kulat biasanya dikesan sebagai komponen utama microorganism yang bertanggungjawab dalam pembentukan gaharu. Walau bagaimanapun, sehingga sekarang formula kulat yang efektif masih belum ditemui. Perbezaan antara gaharu semulajadi dan gaharu buatan adalah berkemungkinan disebabkan oleh perbezaan kepelbagaian kulat yang wujud dalam kedua-dua pokok gaharu. Oleh itu, perbandingan antara kedua sampel ini dari sudut kualiti gaharu dan kepelbagaian kulat adalah perlu untuk mengenal pasti kumpulan kulat yang efektif sebagai penggalak gaharu. Di samping itu, perbandingan tersebut dapat membuktikan kualiti gaharu buatan mempunyai potensi untuk menyamai kualiti gaharu semulajadi berasaskan jangkitan kulat. Kajian dijalankan bertujuan untuk menganalisa kompnen meruap daripada sampel gaharu semulajadi dan buatan, memeriksa dan mengidentifikasi sp.esies kulat yang terlibat dalam penghasilan kayu gaharu daripada kedua-dua sumber. Prestasi inokulan terbaik disaring berdasarkan analisa GCFID selama empat bulan agarwood dengan inokulan komersial masing-masing. Inokulan, Ino A, terbukti merangsang pengeluaran benzaldehyde, 4-phenyl-2-butanone, β-selinene, dan agarospirol. Satu tahun pembuatan kayu gaharu yang dihasilkan secara artifisial (M1 dan M2) menunjukkan pengeluaran sesquiterpene yang efektif seperti agarospirol (4.31% dan 0.301%), β-selinene (0.769% dan 0.643%), aromadendrene (tidak ada dan 1.976%), dan β-agarofuran dan 0.176%) berbanding dengan kayu gaharu semula jadi yang berkualiti tinggi. Pemerhatian makroskopik dan mikroskopik dijalankan ke atas kulat terisolat. *Polymerase chain reaction* (PCR) menggunakan bahagian *internal transcribed spacer* (ITS) daripada isolat kulat digunakan untuk mengenalpasti komposisi komuniti kulat daripada kayu sihat dan kayu cedera daripada kedua-dua sampel ladang dan hutan semulajadi. Berdasarkan analisa, gaharu buatan yang diinduksi selama satu tahun, M1 dan M2, menunjukan penghasilan seskuiterpen yang kompeten seperti agarospirol, β-selinene, aromadendrene dan β-agarofuran, berbanding dengan gaharu semulajadi yang berkualiti tinggi. Agarospirol telah dikesan dalam semua sampel gaharu tetapi kandungan tinggi dalam gaharu buatan. Kayu yang dibuat secara buatan menunjukkan lebih banyak kulat isolat berbanding dengan sampel kayu gaharu asli. Sementara itu, pengasingan kulat dari kayu yang sihat sebagai kawalan menunjukkan pertumbuhan paling banyak enam isolat. Pemerhatian makroskopik dan peperiksaan mikroskopik membolehkan kulat dikumpulkan dalam sepuluh kumpulan. Dalam kajian ini, kulat isolat telah dikenal pasti dari *Lasiodiplodia sp.*, *Aspergillus sp.*, *Fusarium sp.*, *Schizophyllum sp.* dan *Polypolares* Order.Kami membuat penemuan terbaru di mana kulat *Phanerochaete chrysosporum* dan *Schizophyllum commune* telah pertama kali dikenal pasti terlibat dengan kayu gaharu daripada *A. malaccensis* Daripada analisa filogenetik, lima klad taxonomi terbentuk daripada 1000 nilai bootstrap yang menyokong pengenalpastian genomik sebelum ini. Penemuan kami membuktikan bahawa gaharu buatan dapat menghasilkan kualiti gaharu yang hampir setara dengan gaharu semulajadi dan tidak dipengaruhi oleh jenis kulat yang berinteraksi dengan pokok.

ABSTRACT

Aquilaria malaccensis is a tropical tree which produces expensive resinous heartwood (agarwood) through naturally or artificially-induced injury or fungal infection. Fungi are commonly observed as the main microbial component responsible for agarwood formation. Until now, an effective formulation containing fungi-producing agarwood is not reported yet. The difference that exists between natural and artificially induced agarwood may be due to the different fungi diversity. Thus, a comparison between these two samples in terms of agarwood quality and fungi diversity is inevitable in order to determine effective agarwood inducers. Moreover, the comparison may show the quality of artificially induced agarwood to be potentially equal to natural agarwood based on fungal infection. This study focused on the analysis of agarwood volatile compounds from natural and artificially induced agarwood, as well as the isolation, screening, and identification of the fungi species associated with agarwood from both sources. The best inoculant performance was screened based on GCFID analysis of four months induced agarwood with respective commercial inoculant. Inoculant, Ino A, was proven to stimulate production of benzaldehyde, 4-phenyl-2-butanone, β-selinene, and agarospirol. One year artificially induced agarwood (M1 and M2) showed competent sesquiterpene production such as agarospirol (4.31% and 0.301%), β-selinene (0.769% and 0.643%), aromadendrene (none and 1.976%), and β-agarofuran (none and 0.176%) compared to high-quality natural agarwood. Agarospirol was detected in all agarwood samples but higher in artificially induced agarwood. Artificially induced agarwood exhibited more fungi isolates compared to natural agarwood samples. Meanwhile, isolation of fungi from healthy wood as control enabled to grow the most of six isolates from each respective agarwood samples. Macroscopic observation and microscopic examination enabled isolated fungi to be group into ten. It was anticipated that eight groups of fungi isolated were identified in both natural and artificially induced agarwood associated with agarwood formation using culturing and sequencing of ITS regions of the fungi isolated from wood samples. In this study, fungi isolated were identified from *Lasiodiplodia sp.*, *Aspergillus sp.*, *Fusarium sp.*, *Schizophyllum sp.* and *Polypolares* Order. *Phanerochaete chrysosporum* and *Schizophyllum commune* were discovered as new fungi associated with *Aquilaria malaccensis* agarwood. From the phylogenetic analysis, five clades of taxa were formed based on 1000 bootstrap values which supported previous genomic identifications.

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