Molecular Identification and Characterization of *Ganoderma boninense*

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Abstract

*Infeksi **Ganoderma** pada tanaman kelapa sawit merupakan permasalahan utama dalam pengendalian penyakit pada perkebunan kelapa sawit di Indonesia. Sampai saat ini infeksi **Ganoderma** baru diketahui setelah muncul tubuh buahnya pada batang tanaman kelapa sawit, dan pada saat itu tanaman kelapa sawit sudah tak bisa dipertahankan lagi dan tingkat penyebarannya sudah meluas. Penelitian ini bertujuan untuk mengidentifikasi spesies **Ganoderma** yang banyak menyerang tanaman kelapa sawit di Indonesia baik dari segi morfologinya maupun dengan pendekatan biologi molekuler, yaitu berdasarkan pada peta genetik **(sequence)** 18S rRNA dan ITS **(Internal Transcribe Spacer)**. Hasil penelitian menunjukkan bahwa Ganoderma yang banyak menyerang tanaman kelapa sawit di Indonesia adalah **Ganoderma boninense.** Dari hasil karakterisasi telah didapatkan peta genetik spesifik **(specific sequences)** ITS dari **Ganoderma boninense** dibandingkan dengan spesies **Ganoderma** yang lain, sehingga peta genetik spesifik ini dapat dijadikan dasar untuk mendeteksi secara dini adanya serangan **Ganoderma boninense** pada areal perkebunan kelapa sawit di Indonesia. Pendeteksian secara dini terhadap serangan **Ganoderma** ini akan sangat bermanfaat untuk menentukan proses penanganannya dan pada akhirnya diharapkan akan dapat meminimalisasi tingkat kerugian yang terjadi (1).*

Kata Kunci: *Karakterisasi dan identifikasi molekuler **Ganoderma boninense***

I. Introduction

Basal stem rot caused by the Basidiomycete fungus *Ganoderma boninense*, and possibly other species of *Ganoderma*, is the most important disease of oil palm in Malaysia and Indonesia. Yield loss may reach up to 40 % due to the diseases. The fungus initially invades one or more roots and then moves into the stem, causing a dry rot that eventually leads to death of the palm. The disease is currently confined to the coastal areas, where incidence can reach as high as 85 % by the time palms are replanted at 25 years. In planting for jungle or rubber, the disease usually develops when the palms are 10 to12 years old, but on replants from coconuts of oil palm, it can set in as early as 12 – 24 months, but more usually when palms are four or five years old (1)

As with most soil-born diseases, control of the disorder poses major constraints. Clean clearing of the old stand of coconuts and oil palm during replanting has helped, but the incidence in the replants continues to be high to warrant concern. In the existing stands, surgery practiced in the past has been re-introduced in older palms, but the operation is now mechanical. In younger palms, surgery is usually ineffective and the infected palms are removed to control secondary spread of the disease.

Other aspects of the disease control and management

fungicides for their prophylactic and curative properties, bio-control through soil amendments and flood-fallow to weaken or kill the fungus in the soil.

Our approach is to detect or monitor the presence of *Ganoderma boninense* by molecular method, for example, PCR (Polymerase Chain Reaction) and FISH (Fluorescence *in situ* Hybridization). To establish these systems, several *Ganoderma* strains were collected from Northern Sumatera and characterized. The morphological observation indicated that the *Ganoderma* species obtained in Sumatera Island was *Ganoderma boninense*, which will be mainly related to the fungal stem rot disease of oil palm.

II. Material and Merthods

Fungus strains : The fungus strains used in this study are *Ganoderma zonatum*, *Ganoderma boninense* MAFF3-5601, *Ganoderma* strain PTPN-III, *Ganoderma colossum* , *Ganoderma meredithae* and *Ganoderma oregonense*.

***Total DNA dan PCR Reaction*:** The total DNA was isolated from target fungus and the DNA sequences of 18S rRNA of *Ganoderma* strains were amplified by PCR using conserved sequences as primers. The DNA sequencer can analyze approximately 600 – 700 bp long DNA strand. Therefore, we need several PCR products amplified from different positions of rDNA for full length sequencing of 18 S rDNA (1700 bp). We use 7 primers for full sequencing, and any site of rDNA have been analyzed using more than two primers.

Cloning of PCR Products : We used pT7Blue T-vector Kit for cloning of PCR products. pT7Blue vector has been specifically constructed for this application and is provided ready to ligate with amplified DNA. The kit also includes NovaBlue competent cells (*E.coli* strain) for convenient transformation of the ligation products with color visualization of insert-containing clones. This strain has important characteristic of very low reversion frequency to the white phenotype (*lacZ*$^-$) on indicator plates, thus increasing the likelihood of white colonies containing the desired recombinant plasmids.

Bacterial strain and plasmid : *Escherichia coli strain*, which was used for plasmid construction, was grown at 37 °C with shaking in Terrific Broth supplemented with appropriate antibiotics. Whenever necessary, Terrific Broth medium was supplemented with 5 mM IPTG (Isopropanol-β-D-thiogalactopyranoside) (2,3,4). Isolation of Plasmid DNA was prepared based on a modified mini alkaline-lysis/PEG precipitation procedure. Throughout this procedure, the use of a vortex must be avoided so as to minimize shearing of the contaminating chromosomal DNA (5,6).

DNA sequencing : The nucleotide sequences of both strand were determined by the dideoxynucleotide (ddNTP) sequencing methods with single-stranded DNA (7). These strands can be separated by high-resolution acrylamide gel electrophroresis of DNA sequencer. As the fragments pass through a laser beam of sequencer, their fluorescent tags are excited and the emitted is detected by a photomultiplier. Therefore, the length of DNA strands and which base are exists at the end of the strand can be analysis simultaneously. The emission peaks overlap, and a computer analysis is necessary to resolve them. The typical processed output consists of a chart with four tracings, the order of the peaks corresponding to the ladder of the fragments on conventional sequencing gels.

Molecular Identification: To identify the *Ganoderma* at molecular level, 18S rRNA sequence regions were determine, and base on the information of 18S rRNA sequences, phylogenetic relations (trees) of *Ganoderma* were depicted according to NJ method. (Fig.1).

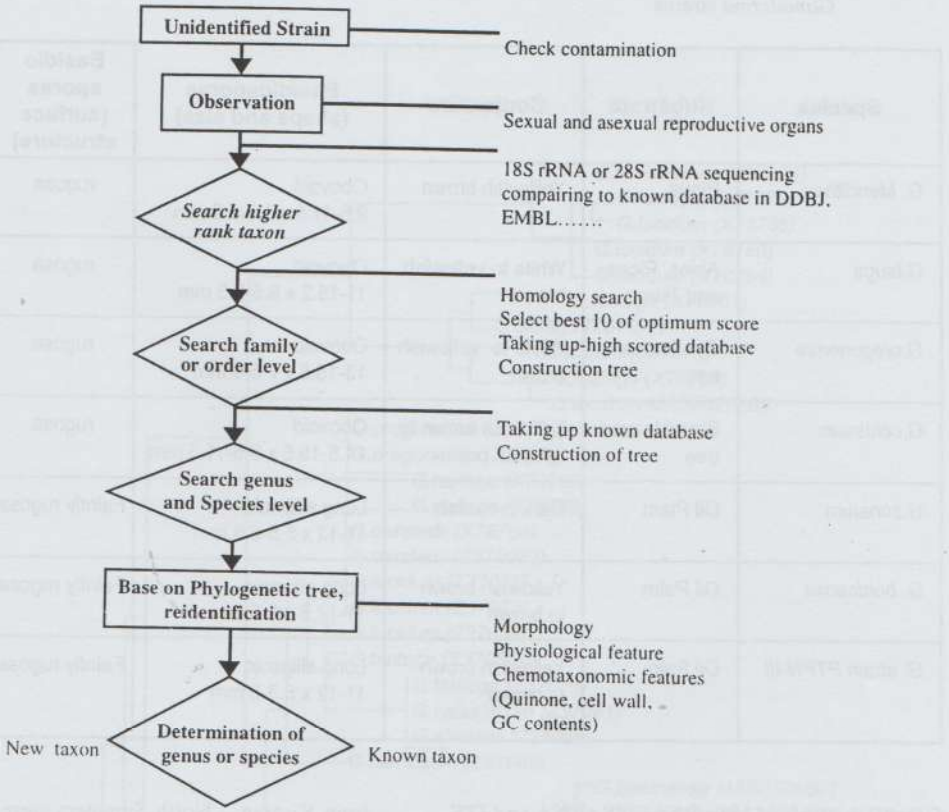

Figure. 1 : Flow chart of Molecular Identification (18S rRNA or 28S rRNA)

III. Results and Discussions

1. Morphological observation of Ganoderma isolated from North Sumatera : The *Ganoderma* strains isolated from palm plantation (PTPN-III) were carefully studied by visual and microscopic observations. As is shown in Table.1, comparing the shape and size of Basidiospores, surface structure of Basidiospores and stripes, the collected strains from PTPN-III were very similar to the *Ganoderma boninense*. Microscopic analyses also confirmed that the strains have the characterics of Ganoderma boninense such as poroid hymenophores, clum connections of hyphae and long ellipsoid and faint rugosa basidiospores (Table.1)

Table.1 : Comparison of Basiodiospores and stripes between *Ganoderma* strain PTPN-III with other *Ganoderma* strains

Species	Substrate	Contexture	Basidiospores (shape and size)	Basidio spores (surface structure)	Stripes
G. Meridithae	Pinus	Yellowish brown	Obovoid 9.5-11.5 x 5.5-6.5 mm	rugosa	stipitate
G.tsuga	Abies, Picea and Tsuga	White to yellowish brown	Obovoid 11-15.2 x 6.5-8.5 mm	rugosa	stipitate
G.oregonense	Coniferous tree	White to yellowish brown	Obovoid 13-15.5 x 7.5-9 mm	rugosa	stipitate
G.colossum	Broad leaved tree	Yellowish brown to grayish brown	Obovoid 14.5-15.5 x 9.5-11.5 mm	rugosa	sessile
G.zonatum	Oil Plam	Dark brownish	Long ellipsoid 11-13 x 5.5-6.5 mm	Faintly rugosa	sessile
G. boninense	Oil Palm	Yellowish brown to brown	Long ellipsoid 11-12,5 x 5-6 mm	Faintly rugosa	sessile
G. strain PTPN-III	Oil Palm	Yellowish brown to brown	Long ellipsoid 11-12 x 5.3-6 mm	Faintly rugosa	Sessile to faint rugosa

Genetic analysis of 18S rRNA. 28S rRNA and ITS sequences : To examine the *Ganoderma boninense* at molecular level, 18S rRNA sequences and ITS (Internal Transcribed Spacers) regions were determined, which is very typical method to identify and classify the microorganisms mycologically. The DNA sequences of 18S rRNA of *Ganoderma* strains were amplified by PCR using conserved sequences as primers and sequenced.

When 18S rRNA sequences of *Ganoderma* isolated from PTPN-III plantation were compared with other *Ganoderma* strains, they were identical to those of *Ganoderma boninense* type strain MAFF305601 (sequence data will be shown elsewhere). This results also confirmed that *Ganoderma* strains isolated I from Kisaran – North Sumatera were *Ganoderma boninense*.. However, from the information obtained above, it is difficult to identify *Ganoderma boninense* from others *Ganoderma* strains. Therefore, the sequences of ITS regions were determined to distinguish between *G.boninense* and other *Ganoderma* strain since ITS regions are known to be more variable at nucleotide level than rRNAs. Based on this information of ITS (internal Transcribe Spacers) sequences, phylogenetic relation (trees) of *Ganoderma* were depicted according to NJ method (Fig.2). A comparison of ITS regions indicated that *Ganoderma boninense* can be distinguished from *Ganoderma lucidum* or other *Ganoderma* strain (data not shown) and specific sequences for *Ganoderma boninense* were identified (data not shown)

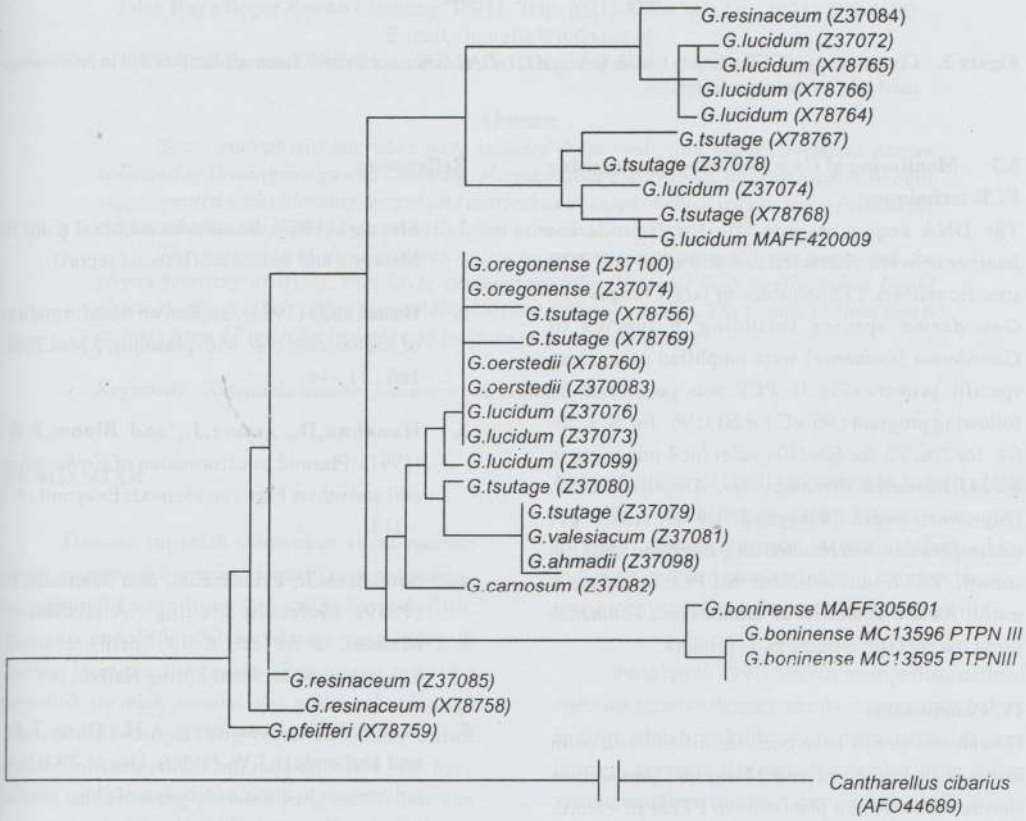

Figure.2 : Phylogenetic relationships of *Ganoderma* based on ITS (Internal Transcribed Spacers) sequences (NJ Method)

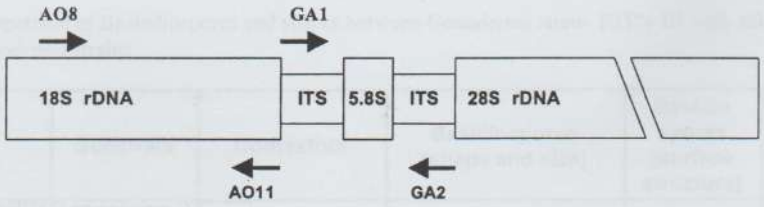

Figure 3. : Construction of PCR primers (AO8, GA1, AO11, GA2 / Data not shown/ Paten application still in processing) used for *G. boninense* detection.

3.3 Monitoring of Ganoderma boninense using PCR techniques:

The DNA sequences specific for *Ganoderma boninense* were characterized and used for PCR specific primers. ITS sequence of target fungus (20 *Ganoderma* species including 1 species of *Ganoderma boninense*) were amplified using these specific primers (Fig.3). PCR was performed by following program ; 96 ∞C for 60 s; 96 for 20 s, 50-64 for 20s, 72 for 60s (40 cycle) for 4 min using in an MJ Research thermocycler. Amplified DNA fragments were analyzed by agarose gel electrophoresis, and resulted only one band (data not shown). This results indicated that PCR technique is useful for the detection of *Ganoderma boninense* using the specific sequences as primers.

IV. Conclusions

Ganoderma strains have been identified as oil palm diseases in Indonesia and Malaysia. *Ganoderma* strains from oil palm plantation in PTPN III - North Sumatera have been isolated. Molecular identification and characterization confirmed that *Ganoderma* strains isolated from PTPN III were *Ganoderma boninense*. The specific sequences of *Ganoderma boninense* have been identified. These spcecific sequences are useful as primers for monitoring or early detection of *Ganoderma* infection using PCR techniques.

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