IJEBAS
International Journal of Economic, Business, Accounting, Agriculture Management and Sharia Administration

MOLECULAR CHARACTERS OF ENDOPHYTIC FUNGI ISOLATE FROM RUBBER LEAVES (Hevea brasiliensis)

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Abstract

Endophytic microbes can be isolated from various types of plants, one of which is from rubber leaves (Hevea brasiliensis). Rubber plants have been planted widely, which means they have the ability to adapt to environmental conditions. Apart from that, currently many rubber clones have been produced which of course harbor potential microbes in them. The potential presence of endophytic fungi can be utilized to support the growth of rubber plants. Isolation of endophytic fungi from rubber plants which have potential as biological agents for controlling diseases, one of which is leaf fall disease. The aim of this research was to determine the diversity of endophytic fungal isolates associated with rubber clone leaves using the DNA extraction method from endophytic fungi from rubber leaves. The results of PCR amplification of the genome DNA of 5 endophytic fungal isolates using primers ITS1 and ITS4 produced one DNA fragment with a size of 612 bp for Aspergillus sp. (BB1) 598 bp for Aspergillus flavus (BB2), 637 bp for Trichoderma asperelum (BB4), 610 bp for Aspergillus sp (KI1) and 621 bp for Aspergillus flavus in isolate KI3 Based on the results of BLAST analysis of the DNA fragment sequence with the fungal isolate sequence Other endophytes in the National Center for Biotechnology Information (NCBI) GeneBank show that the sequence similarity between the isolates analyzed is 99-100%.

Keywords: ***Endophyte, Extraction, PCR, Genome***

1. INTRODUCTION

Isolation of endophytic fungi from rubber plants which have the potential as biological agents for disease control is very important, one of which is leaf fall disease. Currently there are four types of leaf fall disease that attack rubber plants, namely South American Leaf Blight (SALB) leaf fall disease caused by Microcyclus ulei, Colletotricum leaf fall disease caused by C. gloesporioides, Oidium leaf fall disease caused by Oidium heveae and Corynespora leaf fall disease (PGDC) caused by the fungus C. Cassiicola (Atan et al., 2011). In the area of origin of rubber plants, South America, SALB is the main disease that attacks almost all clones so that rubber cultivation is not very developed in that area. Meanwhile, in Southeast Asia, which is the largest natural rubber production center which accounts for more than 90% of world natural rubber production, SALB is not developing, but PGDC is the main threat to decreasing production. In South America, C. cassiicola was found not as a rubber plant pathogenic fungus, but as an endophytic fungus that lives as a saprophyte. So far no symptoms of PGDC have been found, but based on the toxin analysis carried out this isolate has the potential to be a pathogen (Déon et al., 2012).

Molecular identification can be carried out using the ITS (Internal Transcribed Spacer) region of ribosomal DNA (rDNA). DNA isolation and PCR amplification of the ITS rDNA region are the initial steps that need to be carried out in molecular identification. The DNA sequence in the ITS rDNA region evolves more rapidly than other gene regions so it will vary between species

(White et al., 1990). This will make it easier to identify species by comparing the level of similarity (homology) of the DNA sequence of the ITS region of a fungus with that of other fungi.

2. IMPLEMENTATION METHOD

Place and time of research

The research was carried out at the IndoLab laboratory, Jakarta and the DKI Regional Health Laboratory.

Materials and Methods

a. Material

The materials used in this research were PDAS (Potato Dextrose Agar Streptosmycin) media, PDA (Potato Dextrose Agar) media, 1% NaOCl (Sodium Hypochlorite) solution, Polyvinypyroldyne (PVP), CITAB, Sodium acetate, TE buffer, MgCL2, Primer ITS1, Primer ITS4 Rubber Leaf (H. brasilliensis) Clone PB 260, IRR 39 RRIC 100 and PR 261, sterile distilled water, spirit, cotton, 70% alcohol, tissue and cotton wool etc.

b. Tool

The tools used in this research are laminar flow cabinet, autoclave, oven, petri dish, tube needle, Bunsen, gas stove, glass stirrer, case, tweezers, incubator, aluminum foil, microscope, cover glass, object glass, glass. measuring, test tubes, volume pipettes, Erlenmeyer, rulers, media bottles, incubators, analytical scales, scissors, micro pipettes, Watman vacuum discs, No.1 filter paper and others.

Research methods

This research is experimental research, DNA extraction, PCR amplification, electrophoresis and metabolite analysis.

Research Stages

a. DNA Extraction and PCR Amplification of Fungal Endophytes

Pure culture mycelium from selected endophytic fungi was crushed using a mortar and porcelain crusher using liquid nitrogen. DNA extraction was carried out based on the modified method of Castillo et al., (1994), namely by adding polyvinylpirolydone (PVP) to the CTAB extraction buffer and sodium acetate at the DNA precipitation stage. The pellet obtained was washed with 500 µL of 70% ethanol, dried, then resuspended with 100 µL of TE buffer. The DNA suspension is stored at –20°C or can be used directly for further processing. PCR amplification of fungal DNA using the primer pair ITS1 (5'TCCGTAGGTGAACCTGCGG3'), ITS4 (5'TCCTCCGCTTATTGATATGC 3').

The PCR reaction (25 µL volume) consisted of 1 µL sample DNA with a concentration of 25-50 ng µL-1; 18.8 µL nuclease-free water; 2.5 µL 10x PCR buffer (10 mM KCl, 20 mM Tris HCl pH 8.8, 10 mM (NH4)2SO4, 2 mM MgCl2, and 0.1% Triton X-100) (Fermentas, USA); 0.5 µL dNTP 10 mM (Fermentas, USA); 1 µL each of ITS1 and ITS4 primers with a concentration of 10 µM; 0.2 µL recombinant TaqDNA polymerase enzyme 5U µL-1(DreamTaq DNA Polymerase Fermentas, USA). Amplification was preceded by initial denaturation for 5 minutes at 94 °C, followed by 35 cycles through three stages including denaturation for 1 minute at 94 °C, primer

attachment (annealing) for 1 minute at 55 °C, synthesis for 2 minutes at 72 °C C, in the final stage plus 10 minutes at 72 °C. Analysis of amplified DNA was carried out by electrophoresis using 1% agarose gel in Tris Boric EDTA buffer (TBE 0.5x) and visualization using UV light (Sambrook and Russel, 2001).

b. DNA Sequencing Analysis

The amplified DNA was used for the DNA tracing stage based on the dideoxy nucleotide chain termination method (Macrogen Inc., South Korea). The DNA tracing results were then compiled using the Bioedit program and analyzed using the BLASTN program utilizing information from Genbank (http://www.ncbi. nlm.nih.gov).

3. RESULTS AND DISCUSSION

Results of DNA Extraction and PCR Amplification of Endophytic Fungi

Moecular techniques for determining DNA fingerprints of living organisms are an appropriate way to analyze genetic diversity. Results of PCR amplification of genomic DNA of 5 endophytic fungal isolates using primers ITS1 and ITS4 produced one DNA fragment with a size of 612 bp for Aspergillus sp (BB1) (Figure 14), 598 bp for Aspergillus flavus (BB2) (Figure 15), 637 bp for Trichoderma asperelum (BB4) (Figure 16), 610 bp for Aspergillus sp (KI1) (Figure 17), and 621 bp for Aspergillus flavus in isolate KI3 (Figure 18). Based on the results of BLAST analysis of the DNA fragment sequence with sequences of other endophytic fungal isolates in the GeneBank National Center for Biotechnology Information (NCBI), it can be seen that the sequence similarity between the isolates analyzed is 99-100%. Apart from that, BLAST analysis also showed that the DNA fragments obtained included part of the ITS (Interspecific Transcript Spacer) 1 sequence, the complete 5.8S rDNA sequence and part of the ITS 1 sequence.

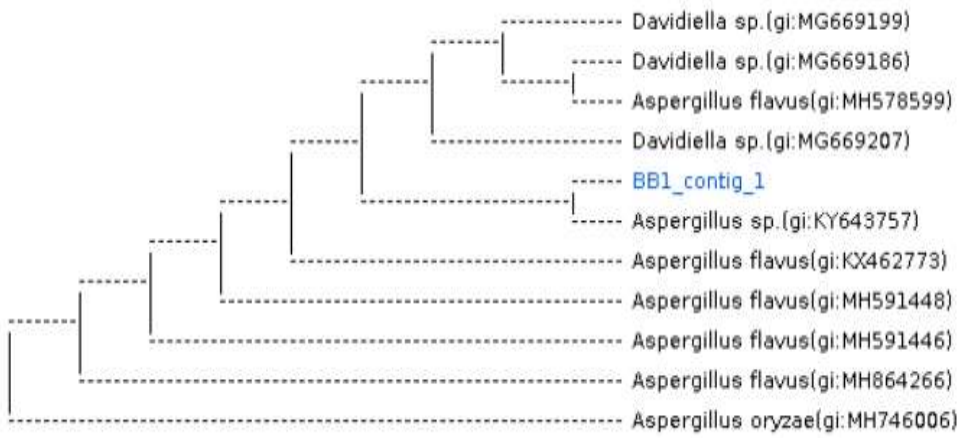

Figure 1. Phylogenetic analysis of endophthal fungal isolates originating from the BB1 rubber clone based on the ITS-rDNA sequence (Aspergillus sp)

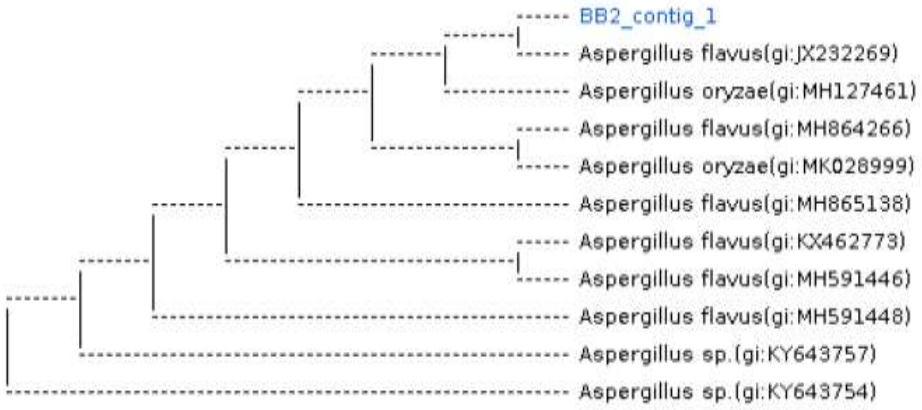

Figure 2. Phylogenetic analysis of endophth fungal isolates originating from the BB2 rubber clone based on the ITS-rDNA sequence (Aspergillus flavus)

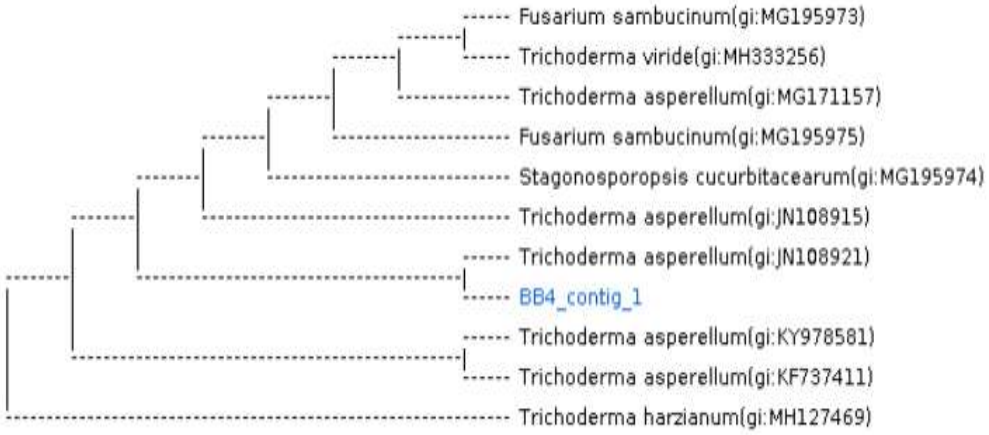

Figure 3. Phylogenetic analysis of endophthal fungal isolates originating from the BB4 rubber clone based on the ITS-rDNA sequence (Trichoderma asperellum)

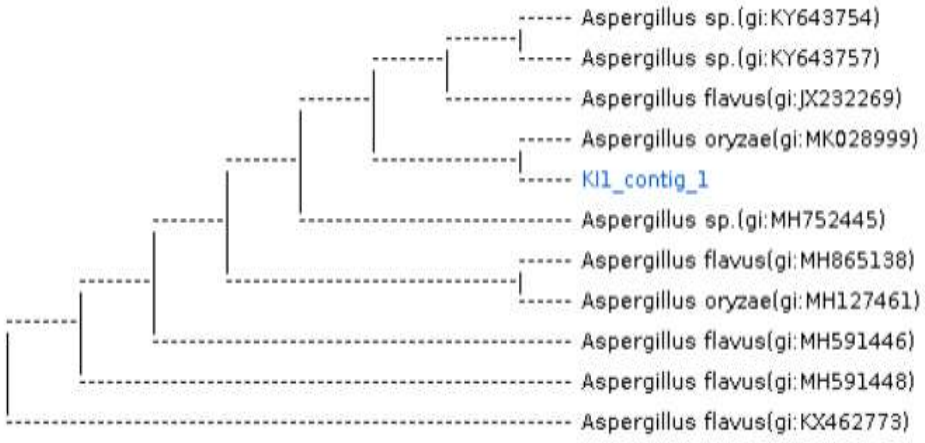

Figure 4. Phylogenetic analysis of endophthal fungal isolates originating from the K1 rubber clone

based on the ITS-rDNA sequence (Aspergillus oryzae)

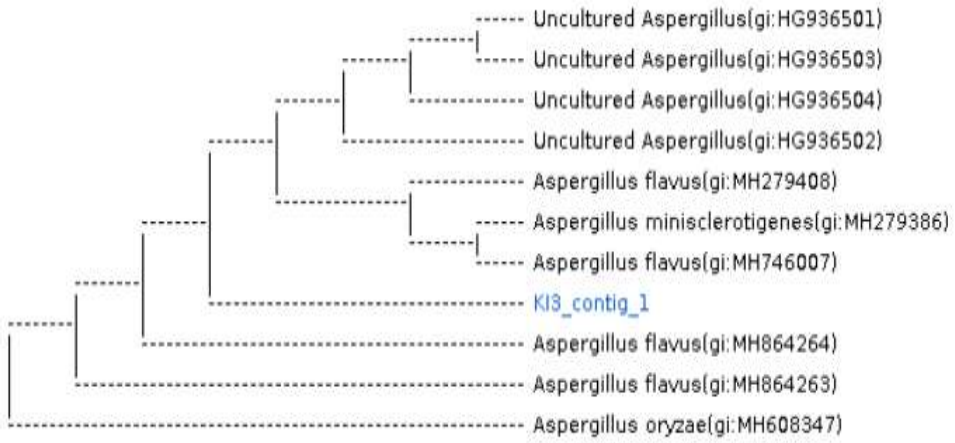

Figure 5. Phylogenetic analysis of endophthal fungal isolates originating from the K3 rubber clone based on the ITS-rDNA sequence (Aspergillus flavus)

4. CONCLUSION

The results of DNA analysis showed that isolates BB1, BB2, BB4 KI1 and KI3 were the fungi Aspergillus sp, Aspergillus flavus, and Trichoderma asperellum, Aspergillus oryzae and Aspergillus flavus, respectively.

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