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Isolation and Amplification DNA on Endophytic Fungi of Local Aromatic Rice Enrekang

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Abstract. Local rice is one of the biodiversity that is currently not explored in detail. The exploration of endophytic fungi of local rice in South Sulawesi was carried out to find out the types of endophytic fungi in the local rice plant. Plant samples were taken from local rice plantations in the Tator, Enrekang, and Luwu regencies. DNA isolation and PCR amplification were the initial stages that need to be carried out in molecular identification. DNA fungi extraction was performed by using DNesay DNA extraction KIT. The result of DNA fungi isolation was electrophoresed with 0.8% agarose gel in TAE buffer (Tris Acetate EDTA). DNA isolation was successfully carried out by the formation of DNA bands. DNA amplification of two endophytic fungi using the primary pairs of ITS1 and ITS4, with condition PCR of pre-denaturation at 95°C for 5 minute, denaturation at 94°C for 1 minute, annealing at 55,4°C for 1 minute, elongation at 72°C for 1 minute, final elongation at 72°C for 10 minute. The denaturation stage until the extension was repeated 35 times and produced DNA fragments with a molecular size of 700 bp and 600 bp.

1. Introduction

Indonesia is the second most biodiverse mega-biodiversity country in the world. This high amount of biodiversity is an invaluable asset that can be used for the welfare of the people. However, the utilization of this natural potential is still constrained by the lack of information and data regarding biodiversity potential.

Microorganisms are one of the biodiversity that needs to be explored in detail to explore its potential in the development of science and technology. Endophytic fungi are one of the microorganisms that have high diversity.

Endophytic fungi are fungi that live in plant tissues without showing symptoms. The highest endophytic concentration is found in crowns, stems, and leaves, while few live in at the roots — mycelia that grow between plant cells, mostly in the protective layer of leaves and reproductive structures. When the host is in the form of seeds, endophytes infect and spread from the plant part of the outer layer into the seed. This shows how endophytes move from plants in a seed production area. When seeds germinate and grow, endophytes infect and spread to the growth tissue of the host plant.

Endophytic fungi can be isolated from various types of plants, one of which is rice. South Sulawesi is one of the local rice-producing regions in Indonesia. Local rice that is quite well known includes Pulu Mandoti from Enrekang, Pare Ambo from Toraja and Tarone from Luwu.



Some endophytic fungi have been isolated from rice plants including; 1) *Nigrospora* sp is an endophytic fungus which most often colonizes at rice stem midribs [1]; 2) Enrekang local rice endophytic fungi that were successfully isolated and morphologically identified are: *Aspergillus* sp1, *Aspergillus* sp2, *Aspergillus candidus* and *Acremonium* [2]; 3) Endophytic fungi have been isolated from rice plants and identified morphologically and have the potential as biocontrol agents from disease: *Fusarium* sp, *Cladosporium* sp, *Phoma* sp, *Penicillium* sp [3].

Identification of endophytic fungi from dahlia tubers has been successfully identified molecularly: *Phialophora stigmata* [4]. Based on the results of the analysis using ITS molecular markers, 4 isolates were identified as genera *Lasiodiplodia*, 4 isolates as *Didymellaceae* family, 10 isolates as the *Phomopsis*, 5 isolates as the genus *Colletotrichum*, 1 isolate as the genus *Nemania*. 1 isolate as genus *Xylaria* [5]. This study aimed to isolate the DNA of local Enrekang endophytic fungi and amplify the isolated DNA.

2. Materials and Methods

2.1. Isolation and purification of Endophytic Fungi

Samples of local rice plants were taken from local rice plants in Enrekang area. Methods of isolating endophytic fungi following the modified Rodriques method [6]. Plant sterilization was carried out at stages by soaking for 60 seconds in 70% ethanol, 3% NaOCl for 60 seconds and 70% ethanol for 30 seconds, then rinsed four times with sterile distilled water and dried on sterile filter paper. Plant parts were cut in small pieces and placed on *potato dextrose agar* (PDA) media and incubated at room temperature. Mycelium that growth on the roots, leaves and stems were grown on new PDA media until pure isolates was obtained.

2.2. Isolation of Fungi Endophytic DNA

DNA fungi extraction was carried out using *DNeasy Plant Mini kit* (QIAGEN) DNA extraction KIT. The extraction process was carried out following the manufacturer's instructions. The fungi were mashed using a hitter. The solution is then incubated in a water bath. The solution was incubated in a water bath for 30 minutes at 65 °C. The solution was centrifuged to separate dirt and supernatant.

2.3. Fungi DNA Amplification Process

The DNA amplification process was started by making PCR mix consisting of hot start PCR mix (QIAGEN), it's 1 and ITS 4 primers, DNA working fungi, and DDh2O. PCR mix solution was included in PCR machines for DNA amplification in vitro. The amplification process consists of initialization/initial denaturation at 95°C for 5 minutes, denaturation at 94 °C for 1 minute, attachment of primer to template DNA (annealing) at 55.4 °C for 1 minute, elongation of 72 °C for 1 minute, and extension of the final 72 °C for 10 minutes. The denaturation stage until the extension then repeated 35 times. DNA amplification process took place for $\pm$ 2 hours 16 minutes.

2.4. Separation of DNA Amplification Process

DNA amplification results were aligned to find out whether the amplification process was successful or not and to determine the size of the amplification product. Separation of DNA amplification results was carried out using horizontal electrophoresis method. This method used 2% agarose and TAE buffer (Tris Acetate EDTA). The separation results were then placed in gel doc to see the results of separation using UV transilluminator and documented.

3. Results and Discussion

3.1. Isolated endophytic fungi

Endophytic fungi isolates which were successfully isolated from the local area of Enrekang rice plants as much as 8 isolates. Based on color and morphology observation items of the colonies, 2 isolates were

selected for further identification. Selected isolates of endophytic fungi were re-grown on new PDA media, and DNA isolation was carried out at the 7th days.

3.2. Isolated of Fungi Endophytic DNA

The process of DNA isolation of endophytic fungi was successfully carried out through agarose gel electrophoresis. The formation of DNA bands on agarose gel observed under UV light showed that the DNA of both Enrekang local rice endophytic fungi had been isolated from mycelia was 7 days old.

3.3. PCR amplification

The use of a suitable primary pair and the right annealing temperature for each fungus is a determining factor in the success of PCR amplification. The primary pair used in this study was ITS1 as a specific fungus primer and ITS4 as a universal primer using an annealing temperature of 55.4°C. The results of the study [7] showed that the PCR process at 58°C for 1 minute gave optimum results. According to [8] the optimum annealing temperature greatly determines the success of the PCR. The high annealing temperature can inhibit the hybridization of the template so that the resulting PCR product is less.

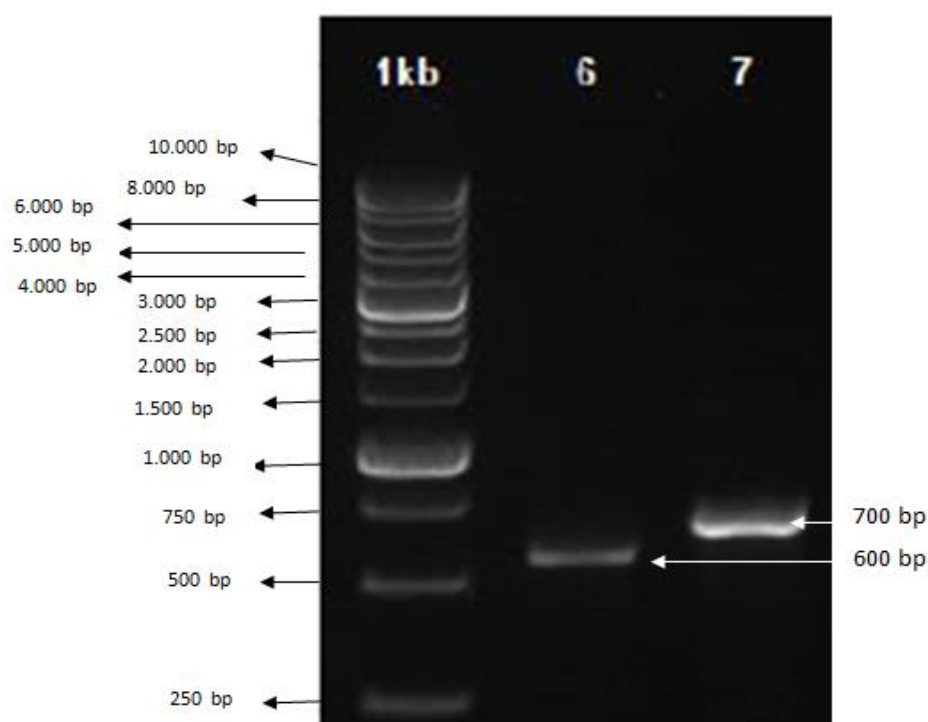


Figure 1. Results of DNA PCR amplification of two local Enrekang rice endophytic fungi isolates using ITS1 / ITS partner 4. 1 kb band path DND Ladder, Line 5 and 6 entophytic fungi DNA bands

The results of DNA amplification of endophytic fungi obtained were 600 bp and 700 bp. In Figure 1, it can be seen that the DNA band obtained is in the form of a single band and indicates that the DNA resulting from the transformation is pure enough.

DNA amplification of fungi using PCR technique often uses the primary pair of ITS1 / ITS4 which will amplify the area of ITS ribosomal DNA (rDNA). Ribosomal DNA (rDNA) is a genome coding region for ribosomal RNA components (RNA). This gene is widely used in phylogenetics, classification, and identification for fungi because of its universal nature, its conservative sequence structure and its presence in large numbers [8].

4. Conclusion

DNA isolation was successfully performed using 0.8% agarose gel electrophoresis characterized by the formation of DNA bands. PCR amplification in the area of ITS-1 and ITS-2 rDNA can be carried out using the primary pairs of ITS1 and ITS4 with an annealing temperature of 55.4 °C and producing DNA fragments of 600 bp and 700 bp

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