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Molecular Characterization Of Phylloplane Mold From Avicennia marina Leaves

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Abstract

Mangroves are a habitat for organisms and microorganisms, including phylloplane molds. Phylloplane molds are known to have various potentials such as antimicrobial, enzyme, and pigment-producing. PFM19 is an orange pigment-producing phylloplane mold. Identification of the mold is needed to determine the species of the fungus so that it can be used for further research. This study aims to identify molecularly the PFM19 mold that produces orange pigment using ITS markers. The methods used in this study included the rejuvenation of isolates, DNA extraction, DNA amplification, and phylogenetic analysis. The results obtained that PFM19 has similarities with Talaromyces islandicus CBS 388.48 by 100% based on ITS markers.

Keywords: *phylloplane mold, molecular characterization, ITS*

INTRODUCTION

Mangroves are in unique wetland forests providing niches for various flora, fauna, and microbes. Mangrove ecosystems are also unique habitats for mold colonization that can produce various types of bioactive compounds In the mangrove area there are a number of environmental stress factors such as high salinity, tides, wind, solar radiation and heat. (Jia *et al*., 2020). In the Mangrove Forest, there are phylloplane molds which are one of the important ecological components (Nayak, B. K., and Anandhu, R., 2017). Phylloplane old is an example of a biological control Pigments are dyestuffs synthesized by plants, animals, and microbes. Pigments have important biological properties, such as antibacterial, antifungal, herbicide, and antioxidant activity that make them essential compounds for a wide range of biotechnology applications (Da Costa Souza *et al*., 2016). Pigments are used by humans to give color to food, clothing, cosmetics, and medicines. Mold pigments become an interesting and important field for research. Many microbes produce pigments in different colors. Some molds include *Aspergillus, Fusarium, Penicillium,* and *Trichoderma* which produce various pigments as intermediate metabolites during their growth (Atalla *et al*., 2011).

. Identifying molds to the species level is very important, this data can be used as a basic consideration for ecological and taxonomic purposes and applications (genomics, bioprospection). These data are important for research related to the role of fungi as a source of bioactive secondary metabolites (Raja *et al*., 2017). One important factor that is likely to hinder progress in research is the difficulty in correctly identifying fungi. This can be done by considering and observing the morphological and molecular characteristics that have been applied in these tasks. Recently, DNA barcodes have emerged as a new method for fast and reliable species identification.

The method that can be used to increase the accuracy of fungal identification is to use a DNA barcode approach, using ribosomal DNA (rDNA) sequences in the ITS (Internal Transcribed Spacer) area. The method that can be used to increase the accuracy of fungal identification is to use a DNA barcode approach, using ribosomal DNA (rDNA) sequences in the ITS (Internal Transcribed Spacer) region (Raja *et al*., 2017, Badotti *et al*, 2017). PFM19 mold is a phylloplane mold that has been isolated in previous studies. In addition, the PFM 19 mold is known to produce orange pigments. Based on Mahardhika *et al*. (2021) PFM19 mold is known to have the ability to produce amylase, protease, and cellulase enzymes. The study also stated that based on the macroscopic and microscopic characterization of morphology, PFM19 has a character like *Penicillium*. Therefore, based on this background, it is necessary to have molecular identification of molds based on ITS

markers to find out the species of the mold, so that it can be further investigated regarding the potential of the mold. This study was conducted to molecular identification of PFM19 phylloplane mold producing orange pigment from mangrove plants..

MATERIAL AND METHODS

Rejuvenation of PFM19 Isolate

PFM19 isolates are isolates of phylloplane mold taken from the *Avicennia marina* mangrove on Mangkang beach, Semarang in the previous study. The isolates were inoculated on PDA (Potato Dextrose Agar) media and then incubated for seven days at room temperature. The mold has grown and is purely ready to be used for the DNA isolation process. DNA extracts of mold cultures were extracted using the CTAB method modified by Aini et al(). Extraction started from the transfer of mold culture to Eppendorf tubes for centrifugation and added 600 µl of CTAB solution. The sample was then incubated for 1 hour at a temperature of 65^{o}C. The tube was then added 600 µl CIA (Chloroform: isoamyl alcohol). The sample was then centrifuged at a speed of 12,000 rpm for 20 minutes. The supernatant phase is separated and transferred into the sterile Eppendorf tube for later centrifugation back. Supernatant added 200 µl isopropanol alcohol for DNA precipitation and incubated 1 hour with a temperature of 20^{o}C. The tube containing the supernatant was then sentenced at 4^{o}C at a speed of 12,000 rpm for 20 minutes to separate the DNA. Pellets are washed by adding 200 µl of 70% ethyl alcohol and dried at room temperature. The washed pellets are then dissolved by adding 50 µl of TE buffer and stored at -20^{o}C.

DNA Amplification and Phylogenetic Analysis

DNA Amplification using ITS5 and ITS4 primers DNA amplification was carried out following the protocol: denaturation at 96^{o}C for 1 minute, annealing at 53^{o}C, and extension at 72^{o}C for 1 minute and 30 seconds. The amplification results are visualized using an electroporator in the electrophoresis process, then the amplification results are sequenced to obtain a nucleotide sequence sample. The sequences are entered in the align process in the Bioedit application to create consensus. Consensus compared to the corresponding sequences in NCBI (BLAST). The phylogeny tree is created using MEGAX with a test-neighbor joining tree and bootstrap method on the jukes-cantor tree model..

RESULT AND DISCUSSION

Based on the results of, the DNA amplification PFM19 mold using ITS4 and ITS5 primers, the size of the ITS area from the mold is 700 bp (Figure 1). This size is optimal for targeting using ITS primers, where the range is 450 to 700 bp. The size of different ITS bands is influenced by the length of the ITS of an organism.

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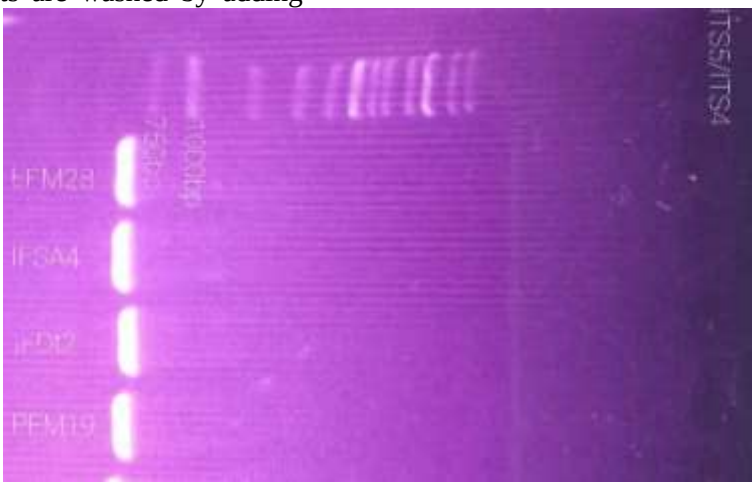

Figure 1. Result of DNA Amplification

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Talaromyces islandicus CBS 338.48 ITS region: from TYPE material	Talaromyces islandicus	1105	1105	97%	0.0	100.00%	644	NR_103664.2
Talaromyces loliensis CBS 643.80 ITS region: from TYPE material	Talaromyces loliensis	1026	1026	97%	0.0	97.83%	636	NR_103680.2
Talaromyces wortmannii CBS 391.48 ITS region: from TYPE material	Talaromyces wortmannii	1000	1000	99%	0.0	96.09%	725	NR_172039.1
Talaromyces radicus CBS 100489 ITS region: from TYPE material	Talaromyces radicus	992	992	97%	0.0	96.83%	636	NR_103666.2
Talaromyces reverso-olivaceus CBS 140672 ITS region: from TYPE material	Talaromyces reverso-olivaceus	989	989	97%	0.0	96.66%	600	NR_171594.1
Talaromyces cerinus CBS 140622 ITS region: from TYPE material	Talaromyces cerinus	983	983	97%	0.0	96.49%	600	NR_169912.1
Talaromyces neorugulosus CBS 140623 ITS region: from TYPE material	Talaromyces neorugulosus	972	972	97%	0.0	96.15%	600	NR_169913.1
Talaromyces ricevillensis NRRL 62296 ITS region: from TYPE material	Talaromyces ricevillensis	944	944	91%	0.0	96.83%	566	NR_155917.1

Figure 2. Result of BLAST NCBI

The results of the phylogenetic analysis, the PFM19 mold has a similarity with *Talaromyces islandicus* with a percent identification of 100% (Figure 3). PFM19 with other species had different percent identification, namely in *T. lowliness*, *T wortmannii*, *T. radicus*, *T. reverso-olivaceus*, *T. cerinus*, and *T neorugulosus* of 97.83%, 96.09%, 96.83%, 96.66%, 96.49%, and 96.15%. Phylogenetic tree analysis also proved that PFM19 has a high bootstrap value with T. *islandicus*. The outgroups used in this analysis of *Saccharomyces cerevisiae*, *A. niger*, and *P. chrysogenum*. PFM19 and the *T. islandicus* isolate are monophyletic groups.

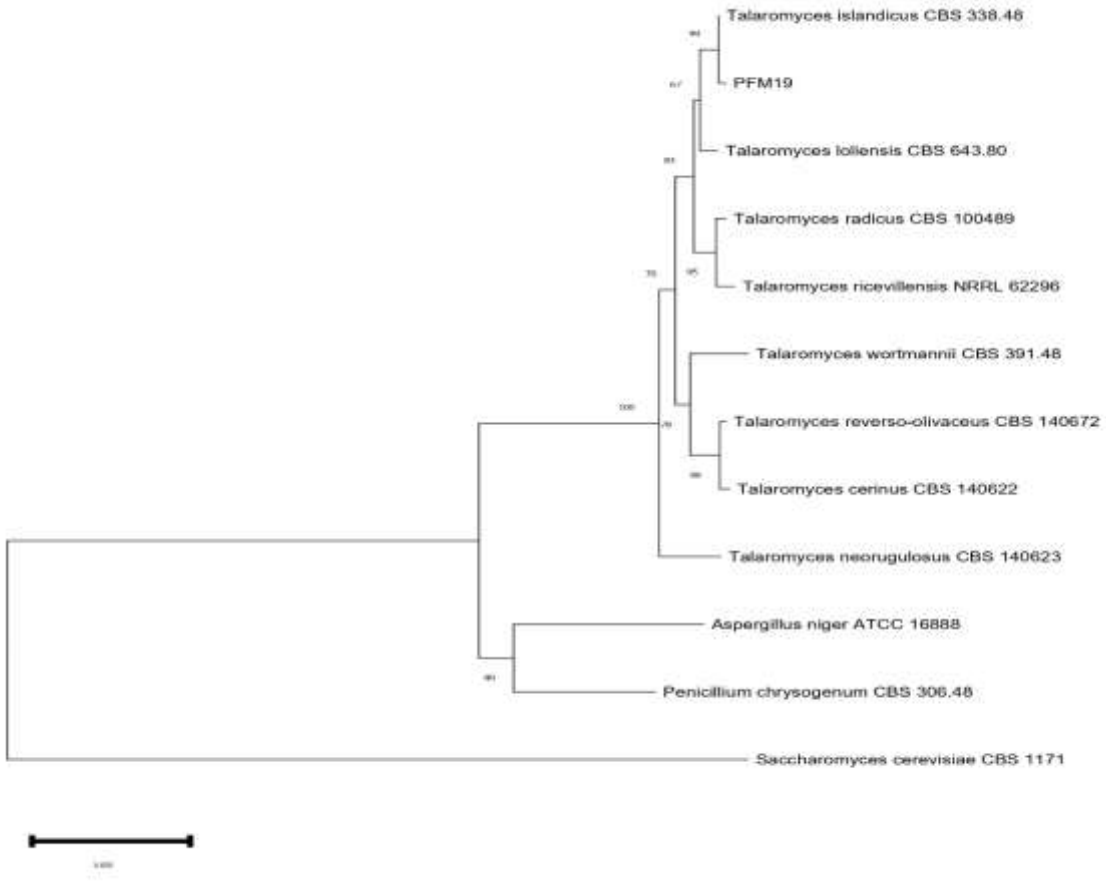

Figure 3. Phylogenetic Tree PFM19

Research by Mahardhika et al (2021) shows that PFM19 morphologically is *Penicillium*. *Penicillium* and *Talaromyces* have a close and similar character. Before 2007, *Talaromyces* was considered the sexual phase of the *Penicillium* or the teleomorph phase, while it was called *Penicillium* if it had not found its sexual phase or anamorphic phase. However, in the most recent classification by Hibbett (2007), both anamorph and teleomorph systems were abolished and from 2011 it was known as the one fungus one name system (Taylor, 2011). *T. islandicus* mold can tether P under conditions of high salinity (Lopez et al. 2020). The mold can also be found as an endophyte in algae. Research by Li et al. (2017) show that *T. islandicus* has the antioxidant activity of hydroanthraquinones. In addition, it is known that the mold also has the Cctn gene, where the gene can produce mycotoxins, namely cychlorotin (Scafhauser et al. 2016)..

CONCLUSION

Based on the results, it can be concluded that PFM19 has a 100% similarity with *T. islandicus*. The results of the analysis are expected to contribute to further exploring the potential of PFM19.

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