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NEW REPORT OF INSECT PATHOGENIC FUNGI (*Aschersonia sp*.) OF CITRUS WHITEFLY (*Dialeurodes sp.*) IN BALI INDONESIA

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Abstract. At the beginning of 2014, in some citrus-producing village in Bangli Regency, Bali, Indonesia, the types of fungi with characteristic yellowish orange to orange was discovered and predicted to be a disease of citrus. To get the correct conclusion of these phenomena, the research about the fungi is needed. Therefore, the study was conducted to identify the fungi base on morphology and molecular characteristic. After a laboratory analysis, it is estimated that the fungi is an entomopathogenic fungus of the genus *Aschersonia* and not a disease of citrus plant. The DNA target of *Aschersonia* between 500-600 bp was success amplified with pair of primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATAT-GC-3'). Based on morphological analysis and molecular identification that includes stroma forms, forms of conidia, and gen similarity with phylogenic analysis, the *Aschersonia* fungi was concluded as *Hypocrella raciborskii* or *Aschersonia placenta*.

Keywords: *Aschersonia, citrus whitefly, insect pathogen.*

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1. Introduction

Citrus is a horticultural commodity that is very popular with the consumers. Citrus are usually consumed fresh or processed in many different varieties. Citrus has a bright prospect of development because of the high economic value (Soelarso, 1996). Unfortunately the development of citrus in Bali in particular often experience problems and decreased production due to pest attacks and diseases. One of the pests is the citrus whitefly. The citrus whitefly causes damage by sucking liquid in the phloem tissue which is the path of nutrition for the plant (Flint, 2015).

In early 2014, in several citrus-producing villages in Bangli District, type of fungi with orange-to-reddish orange color was found. The symptom of the fungi similar with the disease of citrus plant. However, after a laboratory analysis, it is estimated that the fungi is an entomopathogenic fungus of the genus *Aschersonia* and not of plant disease. Base on those data and the potential of *Aschersonia* as an insect pathogen in various places and the absence of information about *Aschersonia* in Indonesia, especially in Bali, the research to identify *Aschersonia* is needed.

2. Materials and Methods

2.1. Aschersonia isolation

The method used in this research based on the method by Liu and Hodge, (2005). First, the samples of infested *Aschersonia* spilled with sterilized water. Let the water be absorbed until the conidia begin to spread. The spreaded conidia then transferred with a sterile needle to the water agar medium with antibiotic. Cultures incubated for 5-6 days then the germinated conidial was transferred to the PDA medium. Then culture on PDA media was incubated for 21 days.

2.2. Morphological identification of Aschersonia

The pure culture of *Aschersonia* was used to analysis the morphological identification by looking at the conidia and the stromal form following the procedure according to Liu *et al*., (2016).

2.3. Molecular Identification of Aschersonia

The molecular identification and analyze started with the DNA extraction. The DNA collected than used for performing PCR using pair of primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATAT-GC-3'). The PCR product was then analyzed using electrophoresis and sequenced to determine the data for the phylogenetic analyze.

2.3.1 Electrophoresis

The 1 µl PCR product (plus 2 µl of loading dye) was electrophoresed in 1% TBE agarose gel. Electrophoresis was carried out for 30 minutes at 100 volts. The DNA that has been electrophoresed is then visualized with a UV translator.

2.3.2. Sequence Analysis

Sequencing have done at Laboratory of First Base, Genetika Science Indonesia for sequence of nucleotides. The sequence results will be analyzed to make the alignment then used to determine the level of homology or alignment with sequence gen *large subunit (LSU) ribosomal RNAA schersonia* that have published in Gen Bank with the Basic Local Alignment Tool (BLAST) Program (NCBI, 2014).

2.3.3 Phylogenetic Analysis

The sequence of nucleotide data then followed by phylogenetic analysis using software ChromasPRO, Molecular Evolutionary Genetics Analysis (MEGA 5.05), PAUP, BioEdit, and TreeGraph2.

3. Results and Discussion

3.1. Aschersonia isolation

Isolation of insect pathogens *Aschersonia* was performed to obtain pure *Aschersonia* culture in PDA media. This pure culture is very important for identification. Isolation of *Aschersonia* following the method by Liu and Hodge (2005),has successfully done to get a pure culture of *Aschersonia.*

3.2. Morphological identification of Aschersonia

The identification of *Aschersonia* was following the identification method according to Liu *et al.*, (2006) and Wirya *et al.*, 2016. The identification method used in this study was based on the form of conidia and the shape of stroma.

3.2.1 Shape and color of sexual stroma

The shape and color of this stroma can be directly observed in the field and usually located on the underside of the leaf. When observed the shape and color of the stroma from samples collected from the field, the *Aschersonia* samples from Jehem Village has the most morphological characteristics close to *Aschersonia aleyrodis* and *Aschersonia placenta* (Figure 1).

Figure 1. Comparison of forms of *Aschersonia* sexual stroma. A. *Aschersonia* from the field, B. *Aschersonia placenta* (Liu *et al.*, 2006), C. *Aschersonia aleyrodis* (Liu *et al.*, 2006)

Aschersonia aleyrodis and *Aschersonia placenta* are two species of *Aschersonia* that have almost similar characteristics and are quite difficult to distinguish morphologically (Wirya *et al.*, 2016). *Aschersonia aleyrodis* and *Aschersonia placenta* both have a flat-shaped stroma shape. The conidia produced during the stroma of *Aschersonia aleyrodis* and *Aschersonia placenta* also have similarities that are orange to yellowish orange (Liu *et al.*, 2006).

3.2.2. Form of Conidia

Through microscopic observation of the conidial form of the samples obtained in the field and the form of conidia culture on PDA media showed the same results. The similarity of conidia form between samples and culture results indicates *Aschersonia* that growing on PDA is the same *Aschersonia* found in the field (Figure 2).

Observations of the conidial forms obtained from samples and cultures on PDA then matched with the reference used for identification. The results showed fusoid conidia with a length of 9-16 μm and width of 1.5-2 μm similar to character of *Aschersonia aleyrodis* and *Aschersonia placenta* (Liu *et al.*, 2006; Wirya *et al.*, 2016). This similarity to both *Aschersonia* species confirmed that this identified *Aschersonia* is one of the two species. *Aschersonia aleyrodis* and *Aschersonia placenta* are two species of *Aschersonia* that have almost identical characteristics and are quite difficult to distinguish morphologically. The morphological result has not been clear conclusion to identified of *Aschersonia*, therefore the molecular identification is needed to correct result of identification.

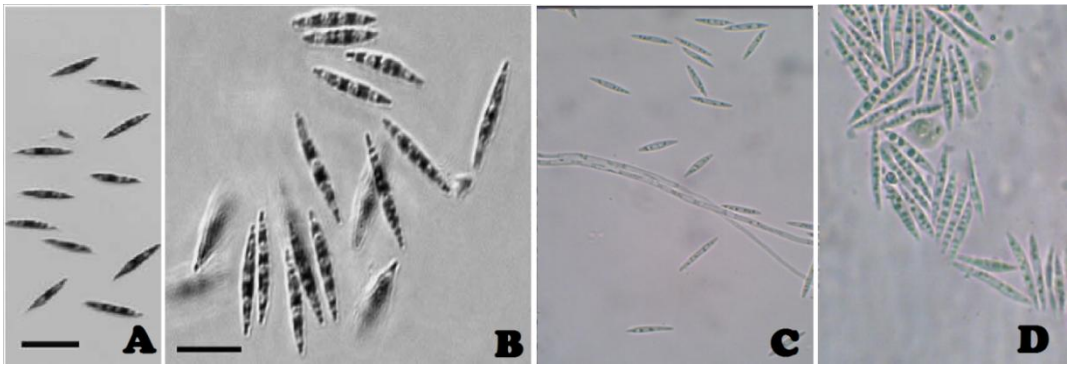

Figure 2. Comparison of conidial forms of *Aschersonia*. A. Conidia of *Aschersonia aleyrodis* (Liu *et al*., 2006), B. Conidia of *Aschersonia placenta* (Liu *et al.*, 2006), C. Conidia of samples taken in the field, D. Conidia of *Aschersonia*'s culture growing on PDA media (Wirya *et al.*, 2016)

3.3. Molecular Identification of Aschersonia

Molecular analysis of the *Aschersonia* samples from Jehem (AJR1) has been carried out successfully in several stages. DNA extraction using ZR-based Fungal/Bacterial DNA Kit ™ Catalog No. D6005 by ZYMO RESEARCH has managed to get results as needed. The results of *Aschersonia* DNA extraction were then performed PCR using universal primers ITS-1 and ITS-4. The product from PCR was then electrophoresed and sequenced then the data obtained was analyzed to get the desired information.

3.3.1 Electrophoresis

The PCR product obtained and electrophoresed showed the DNA band that appeared. The amplified DNA band showed a base length between 500-600 bp (Figure 3). The emergence of DNA bands is an important information that shows that PCR has been successfully carried out on the DNA sample believed as *Aschersonia*. However, these results have not been able to ascertain the truth about whether the amplified is truly DNA from *Aschersonia* because the primers used is an universal primers. Therefore, sequencing is carried out to ensure the correctness of the information needed in this study.

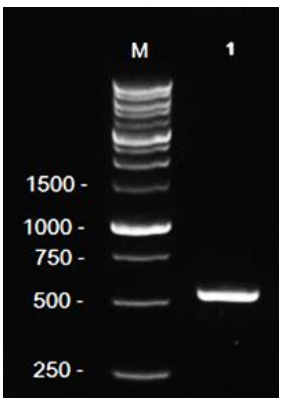

Figure 3. PCR product of gen target of *Aschersonia*. M is marker and lane 1 is *Aschersonia* samples from Jehem (AJR1)

3.3.2 Phylogenetic Analyze

The results of phylogeny analysis on the sequences of AJR1 divide the sequences into several groups. *Aschersonia* sample (AJR1) then formed a wider branching with group of *Hypocrella raciborskii* or *Aschersonia placenta* and indicating that AJR1 is fungi from the genus *Aschersonia* and the species is *A. placenta* (Figure 4).

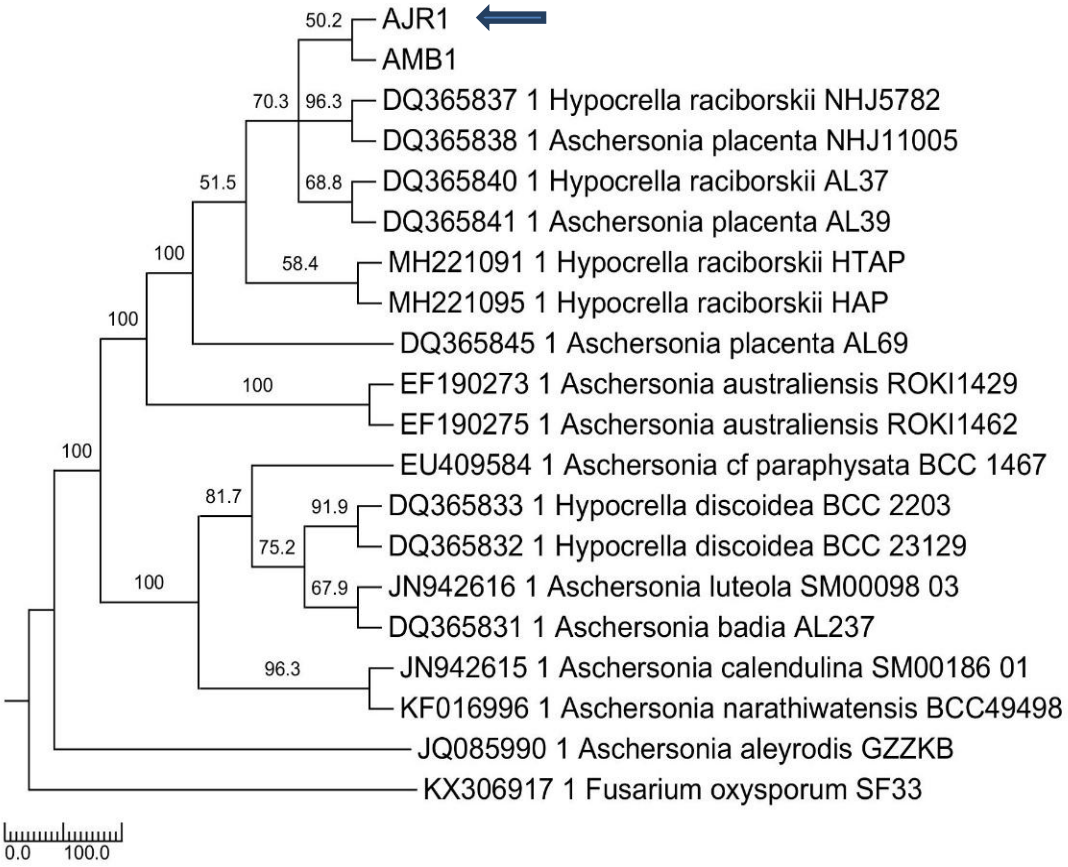

Figure 4. Phylogenetic tree arranged based on DNA composition of AJR1 with the Maximum Parsimony method. The number in the branch is the percentage of the level of trust in the group. Arrow indicated the sample of Bali *Aschersone* sequence

Based on those identification results, it was concluded that the species of insect pathogen identified as *Aschersonia placenta*. Liu *et al.*, (2006) states that *Aschersonia aleyrodis* has never been previously reported to be found in Indonesia, even in Southeast Asia. While *Aschersonia placenta* known distributions include: Cameroon, China, Ghana, India, Indonesia, Malaysia, New Guinea, Philippines, Thailand, and Vietnam (Liu *et al*., 2006).

In addition, *Aschersonia* was utilized and found widely as insect pathogen of whitefly in the word since long time ago (Petch, 1921; Ramakers & Samson, 1984; Liu *et al.*, 2006). Therefore the *Aschersonia* is very potensial agent to control insect pest especially whitefly in Bali. The new information about *Aschersonia* is useful for mass production of *Aschersonia* as a biological control of insects pest.

4. Conclusion

Based on those identification results, it were concluded that the sample of the fungi is insect pathogen member of genus *Aschersonia* and the species is *Aschersonia placenta*.

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