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Research Article

Fusarium equiseti Isolated from *Lysurus periphragmoides* Fruiting Body

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Abstract

Phyllosphere fungus is a fungus that attaches or moreover enters a tissue of the host. Mostly, the fruiting body that grows on the soil or other substrate openly can be colonized by the phyllosphere fungi. *Lysurus periphragmoides* was found on the soil. The fruiting body was opened on the head part and the holy structure on the stem part. Fungal isolation from the stem was conducted with surface sterilization before cultivating onto PDA medium. The filamentous fungus (isolate Lyz111) was appeared on isolation medium and identified as *Fusarium equiseti* using multigene analysis (ITS and LSU regions). This is the first report that *Fusarium equiseti* have been found as phyllosphere fungus on *Lysurus periphragmoides* fruiting body.

Keywords: Filamentous Fungus; *Lysurus periphragmoides*; Multigene; Phyllosphere Fungus

Abbreviations

ITS and LSU

Introduction

Fusarium incarnatum-equiseti species complex (FIESC), one of 17 species complexes of the genus *Fusarium* [1], has a few formally described species that are characterized by dorsiventral curvature of macroconidia and abundant chlamydospores [2]. This species complex consists of more than 30 phylogenetic species [1-6] and the members are ubiquitous, some of them take a role as pathogens, saprobes, or secondary invaders [3,7]. One of the members of FIESC is *Fusarium equiseti*. *Fusarium equiseti* was considered a weak pathogen [8] even considered as an unimportant plant pathogen and a cosmopolitan soil inhabitant [9,10]. However, this species still can attack various crops and was recently reported causing Chilli wilt in Kashmir, Northern Himalayas [11]. *F. equiseti* may produce a variety of toxic secondary metabolites including diacetoyxyscirpinol, moniliformin, and 8-O-methylbostrycoidin [12]. *Fusarium equiseti* is grouped by O'Donnell, Sutton, Rinaldi, Gueidan., *et al.*"mendeley":{"formattedCitation":"(O'Donnell, Sutton, Rinaldi, Gueidan, et al. [3] in FIESC 14-a. It is also found on substrates or hosts in the form of soil, potato peel, maize husk, hordeum vulgare, Daphne mezereum, sediment, human toenail, and *Beta vulgaris* [13]. With all the information, the information about *Fusarium equiseti* substrate or host is still lacking. Thus, this study aimed to describe the morpho-molecular *Fusarium equiseti* isolated from the fruiting bodies of *Lysurus periphragmoides*.

Materials and Methods

Fungal Isolation

The substrate was *Lysurus periphragmoides* fruiting body [14]. The stem part of *Lysurus periphragmoides* (Figure 1) was used to isolate the fungi. The surface sterilization was conducted following the protocol from Greenfield., *et al.* [15]. The stem part of *Lysurus* fruiting body was sliced carefully in a sterile condition. The part was washed using the surface sterilization solutions, such as sodium hypochlorite (0.5%), ethanol (70%), and sterile distilled water. The *Lysurus* parts were dried using sterile papers for 1 night in laminar airflow. The *Lysurus* parts were put on Potato Dextrose

Agar (PDA) with modification as half potato composition of usual composition. The tissues were incubated at 28 C for 4 - 7 days. The mycelia that grew on the medium was transferred into a fresh PDA (normal composition).

Morphological identification

The mycelium was observed in the PDA medium. The characters such as spore, somatic hyphae, and colony appearance were observed using microscope binocular microscope cs22LED. All characters looked like Hypomycetes fungi or Mitosporic Fungi. Therefore, the morphological identification used special literature book as mitosporic fungi identification book from Kiffer and Morelet [16].

Molecular Identification

The fungal genomic DNA was extracted using the DNA extraction protocol in Hermawan., *et al*. [17]. The cetyltrimethyl-ammonium bromide (CTAB) solution was used in this study as a lysis solution. The quality and concentration of the DNA were measured using a spectrophotometer nanodrop. The good quality DNA was used to be continued for amplification. The concentration was around 100 ng/µl. Then, the DNA was amplified using two primers, i.e. internal transcribed spacer (ITS) and large subunit (LSU). The ITS primers were ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS 5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3'). The LSU primers were LR0R (5'-GTA CCC GCT GAA CTT AAG C-3') and LR5 (5'-ATC CTG AGG GAA ACT TC-3'). PCR amplification was conducted in a 40 µL total reaction. The PCR mixture was composed as 20 µL PCR mix of 2X Kappa Fast 2G, 2 µL of 10 pmol of each primer, 4 µL 100 ng template DNA, and 12 µL ddH_2O. A Thermoline PCR was used for the DNA amplification. The PCR condition was set as follows: initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 45 seconds, annealing at 55°C (for ITS primer) or 56°C (for LSU primer) for 1 minute, extension at 72°C for 1 minute, and the final extension at 72°C for 10 minutes. The cycles from denaturation to the extension were repeated for 30 cycles. The amplicon was estimated on 1% agarose gels and visualized by the Gel DocTM XR system. PCR products were sent to the 1st Base Malaysia company for sequencing using Sanger dideoxy method.

Figure 1: Stem part of *Lysurus periphragmoides* fruiting body.

The sequences were assembled using the ChromasPro application. The assembled sequences were deposited into GenBank (https://www.ncbi.nlm.nih.gov/). The sequences were continued to do Basic Local Alignment Search Tool (BLAST) using the NCBI website. The genera will be shown after the BLAST processing. Then, all sequences of Fusarium species (Table 1) were downloaded using current literatures as sequence references [18,19]. Multigene analyses were used to build the phylogenetic tree. The genes of sequences were compiled using MEGA X software which the ITS gene as the first gene and the LSU gene as the second gene. The compiled genes were processed in Clustal X software to get the Phyllip format file [20]. The phylogenetic tree reconstruction used Randomized Axelerated Maximum Likelihood (RAxML) Black Box was generated on CIPRES [21]. The Bootstrap analyses as 1000 replications were used in this phylogenetic tree reconstruction. The Bootstrap (BS) value ≥ 75 was shown on the branch.

Species	Isolate Code	GenBank Accession Number	
		ITS	LSU
Aspergillus niger	DAOM 221143	JN942866	JN938930
Fuasrium proliferatum	CBS 143592	LT970804	LT970804
Fusarium acuminatum	CBS 143612	LT970802	LT970802
Fusarium equiseti	CBS 307.94	MH862468	MH874117

Fusarium equiseti	CBS 219.63	MH858268	MH869877
Fusarium equiseti	CBS 126202	MH864013	MH875469
Fusarium equiseti	Lyz111	OM345041	OM345040
Fusarium gamsii	CBS 143610	LT970824	LT970824
Fusarium gamsii	CBS 143609	LT970823	LT970823
Fusarium iranicum	CBS 143608	LT970821	LT970821
Fusarium iranicum	CBS 143611	LT970822	LT970822
Fusarium oxysporum	CBS 143607	LT970803	LT970803
Fusarium oxysporum	SJB143	LC633891	LC633904
Fusarium oxysporum	SJB232	LC633892	LC633905
Fusarium proliferatum	CBS 143594	LT970805	LT970805
Fusarium proliferatum	CBS 143599	LT970806	LT970806
Fusarium solani	SJB222	LC633898	LC633911
Fusarium solani	SJB243	LC633899	LC633912
Fusarium solani	SJB62	LC633897	LC633910

Table 1: Species, Isolate Code and GenBank Acc. Number in this study.

Results and Discussion

Isolate Lyz111 was identified as Fusoid fungal group based on the morphological observation (Figure 2). The colony was cottony and whitish milk color. The spore mass looks like pale cream to dark brown. The isolate forms a pale brown to dark brown pigment on PDA medium. The macroconidia were observed on the PDA medium. The microconidia were not observed until 30 day-incubated days. The macroconidium was fusoid shape with 4 to 6 septate. Macroconidium was smooth on the surface and hyaline. Clamydospore was observed when the incubation age was in 21 days more. The macroconidium was 4.2-4.6 µm in width and 25.2-30.4 µm in length. The isolate Lyz111 was isolated by Rudy Hermawan. The Lyz 111 GenBank accession numbers were OM345041 for ITS gene and OM345040 for LSU gene.

Figure 2: Fusarium Lyz111 isolate on PDA medium for 7 days incubation. (A) Colony on 6 cm diam. Petri Dish; (B; C) macroconidia. Scale bars: (B) 30 µm; (C) 5 µm.

Based on the morphological observation, the spore had the fusoid shape. It can be known as the Fusarium as one of the possible genera for the Lyz111 scientific name. To identify the *Fusarium* until species, recently it needs more than one gene [18,19,22,23]. Internal Transcribed Spacer (ITS) is the popular and important gene to identify the micro-fungal species [24]. This study used ITS as the first identification gene, then Large Subunit (LSU) as the second gene. The LSU gene was used in Hermawan and Khairillah [20] to identify the *Xylaria*. But the result of them showed that the LSU gene was not enough strong to identify their fungus until the species name. In our study, we combined the ITS and LSU to make a multigene phylogenetic tree.

The multigene phylogenetic tree of *Fusarium* species including the Lyz111 isolate (Figure 3) showed that the Lyz111 isolate was identified as *Fusarium equiseti* with 77 BS value. This value is enough strong to make the Lyz111 isolate as *Fusarium equiseti* clade. The sister clade of *Fusarium equiseti* is *Fusarium oxysporum*. The molecular identification of *Fusarium* was popular to use many genes for species identification, such as Elongation Factor (Tef), Large Subunit (LSU), RNA polymerase II gene (RPB2), Calmodulin (CAM), and Internal Transcribed Spacer (ITS) [18,19,22,23]. The multigene analysis can show the strong clade structure among the species if as compared with the single-gene analysis.

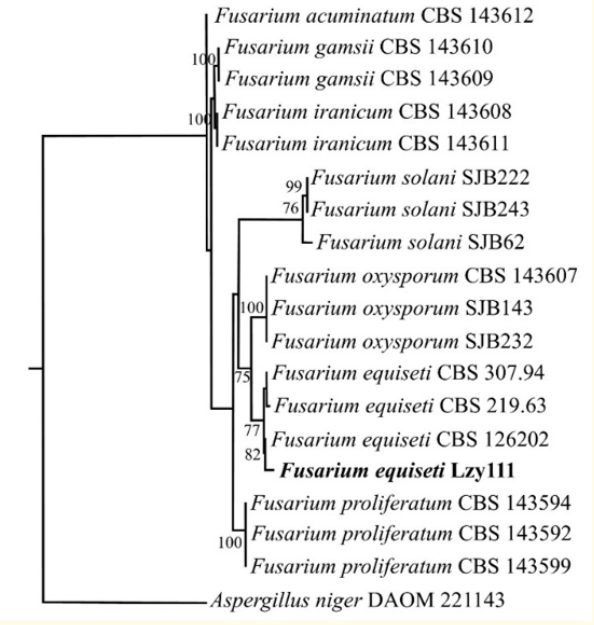

Figure 3: Fusarium phylogenetic tree based on the ITS4/ITS5 and Large Subunit regions (multigene analysis) using RAxML. Bootstrap (BS)≥75 was shown on the branch. The Fusarium strain Lyz111 must be in bold.

The isolate Lyz111 as *Fusarium equiseti* could not produce the microconidia on PDA medium. This is supported by the description from Leslie and Summerell [2]. Distinctive from other *F. equiseti* from Hami., *et al*. [11] that can produce microconidia on PDA medium. *Fusarium equiseti* is a cosmopolitan soil that can inhabit some organisms from soil habitat. Therefore, it is possible for *Lysurus* species is colonized or attached by *F. equiseti*. Some organisms were also potentially infected by *F. equiseti*, such as animal. *F. equiseti* is reported can cause some poisoning cases to cattle, chickens, ducklings, guinea pigs, mice, pigeons, pigs, rabbits, and rats [25]. Some studies described the toxins that can be produced by *F. equiseti*, such as butanolide [25], beauvericin [26], equisetin [25,27], fusarochromanone [28], and zearalenone (25 Marasas., *et al*.).

In the plant, *F. equiseti* can be as a saprophytic fungus in root, stem base, and seed part [2]. However, in this study the *F. equiseti* Lyz111 isolate was found as the phyllosphere fungi in other fungus (*L. periphragmoides*). This is the first record in *F. equiseti* ecological characteristic. But the role as phyllosphere fungus in other fungus does not investigate more comprehensive in this study. The role can be a saprophytic, pathogenic, or moreover as endophytic fungus. The continued research about this role is needed to confirm it sooner.

The main purpose for this isolation is to get the *Lysurus* mycelium in culture medium. But as a result, the *Lysurus* cannot isolate successfully. The *Fusarium equiseti* colonies were appeared in our replication and repetition of isolation. The *Fusarium* was easy to grow faster than the main fungus (*Lysurus*). Despite the surface sterilization was conducted to minimize the phyllosphere microorganisms harboring during the isolation process. The *Fusarium equiseti* might enter the *Lysurus* stem tissue. So, the *Fusarium* can be survived and isolated into the medium after surface sterilization. Another mushroom such as *Xylaria*, Hermawan, Safitri., *et al*. [21] also reported that micro-fungus as *Neopestalotiopsis zimbabwana* colonized the stromata even the surface sterilization done.

Conclusion

Isolate Lyz111 isolated from *Lysurus periphragmoides* stem part was identified as *Fusarium equiseti* by multigene analysis and supported by morphological identification. This is the first report that *F. equiseti* was found in *L. periphragmoides* fruiting body as phyllosphere fungus.

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