

Saprophytic and Potentially Pathogenic *Fusarium* Species from Peat Soil in Perak and Pahang

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Abstrak: Pencilan *Fusarium* telah dipencilkan daripada sampel tanah gambut yang diperolehi dari hutan paya gambut, tanah gambut takung air, dan tanah gambut ladang kelapa sawit. Pencirian secara morfologi digunakan untuk mengenal pasti pencilan tersebut secara tentatif dan penentuan spesies berdasarkan jujukan gen faktor pemanjangan translasi-1 α (TEF-1 α) dan analisis filogenetik. Berdasarkan padanan yang terdekat carian *Basic Local Alignment Search Tool* (BLAST) pangkalan data Genbank dan Fusarium-ID, lima spesies *Fusarium* telah dikenal pasti iaitu *F. oxysporum* (60%), *F. solani* (23%), *F. proliferatum* (14%), *F. semitectum* (1%), dan *F. verticillioides* (1%). Daripada analisis filogenetik penyambung jiran menggunakan gabungan jujukan gen TEF-1 α dan β -tubulin, menunjukkan pencilan dari spesies yang sama dikelompokkan dalam klad yang sama walaupun variasi intraspesies diperhatikan melalui analisis filogenetik. Spesies *Fusarium* yang dipencilkan dalam kajian ini adalah penghuni tanah dan tersebar meluas di seluruh dunia, dan boleh menjadi saprofit dan pengurai. Kehadiran spesies *Fusarium* dalam tanah gambut mencadangkan tanah gambut boleh menjadi takungan patogen tumbuhan di mana beberapa spesies patogen tumbuhan terkenal seperti *F. oxysporum*, *F. solani*, *F. proliferatum*, and *F. verticillioides* telah dikenal pasti. Keputusan kajian ini juga memberikan pengetahuan tentang kemandirian dan penyebaran spesies *Fusarium*.

Kata kunci: *Fusarium*, Tanah Gambut, Morfologi, Filogenetik

Abstract: Isolates of *Fusarium* were discovered in peat soil samples collected from peat swamp forest, waterlogged peat soil, and peat soil from oil palm plantations. Morphological characteristics were used to tentatively identify the isolates, and species confirmation was based on the sequence of translation elongation factor-1 α (TEF-1 α) and phylogenetic analysis. Based on the closest match of Basic Local Alignment Search Tool (BLAST) searches against the GenBank and Fusarium-ID databases, five *Fusarium* species were identified, namely *F. oxysporum* (60%), *F. solani* (23%), *F. proliferatum* (14%), *F. semitectum* (1%), and *F. verticillioides* (1%). From a neighbour-joining tree of combined TEF-1 α and β -tubulin sequences, isolates from the same species were clustered in the same clade, though intraspecies variations were observed from the phylogenetic analysis. The *Fusarium* species isolated in the present study are soil inhabitants and are widely distributed worldwide. These species can act as saprophytes and decomposers as well as plant pathogens. The presence of *Fusarium* species in peat soils suggested that peat soils could be a reservoir of plant pathogens, as well-known plant pathogenic species such *F. oxysporum*, *F. solani*, *F. proliferatum*, and *F. verticillioides* were identified. The results of the present study provide knowledge on the survival and distribution of *Fusarium* species.

Keywords: *Fusarium*, Peat Soil, Morphology, Phylogenetic

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INTRODUCTION

The genus *Fusarium* is one of the most important groups of Ascomycetes fungi. They are well-distributed soil inhabitants with the ability to be parasites or saprophytes. The majority of *Fusarium* survive in plant debris and live close to the soil surface (Nwanma & Nelson 1993). *Fusarium* species have special characteristics, such as chlamydospores and resistant conidia, which help support their survival in soils and plant debris.

Peat soils can be obtained from peat land in forests, peat land under crops, and waterlogged peat swamps. Peat land in forests and peat land under crops provide dry areas, while waterlogged peat swamps provide wet areas for *Fusarium* habitation. These three types of peat soils share similar characteristics, such as acidic environments (pH 2–4), high moisture content, high carbon content, and low nitrogen content. Most of the tropical peat lands in Malaysia are geologically recent, as most of the areas were formed over the past 5,000 years (Yule & Gomez 2009).

Peat lands are formed by poor drainage, permanent water logging, and slow decomposition of the organic matter in acidic environments due to rainfall and topography. Most lowland peat contains partially decomposed trunks, branches, and roots of trees (Rieley *et al.* 1996), which result in low nutrient content in soils due to litter build-up as peat (MacKinnon *et al.* 1996). Peat soil in peat swamps were formed by the accumulation of partially decaying plant debris in waterlogged conditions, high levels of acidity (pH 2.5–4.7), and lack of oxygen. These conditions prevent microorganisms from rapidly decomposing the plant debris (Pinruan *et al.* 2007). Peat swamp forests are peat land tropical forest and are rapidly being depleted due to conversion into agricultural land and logging.

The genus *Fusarium* was one of the fungal genera listed in the compilation of fungi from boreal peatland ecosystems (Thormann & Rice 2007). From a preliminary study conducted by Latiffah *et al.* (2010), *Fusarium* species were detected in peat land areas in Perak, Malaysia. Because *Fusarium* can survive in this extreme environment, the peat ecosystem might be a reservoir for pathogenic *Fusarium* species. Moreover, many peat land areas are converted to agricultural land, which might provide suitable growth conditions for many species of *Fusarium*. Information on the presence of various species of *Fusarium* in peat soil is important, as many *Fusarium* species are soil-borne pathogens and some plant pathogenic species are cosmopolitan. Therefore, the objectives of this present study are to determine the presence of *Fusarium* species in peat soils as well as to characterise *Fusarium* isolates using phylogenetic analysis.

MATERIALS AND METHODS

Soil Sampling and Isolation

Peat soil samples were collected from Perak (Sungai Beriah and Beriah Kecil, Bukit Merah) and Pahang (Sungai Bebar, Pekan) in Peninsular Malaysia. In Perak, soil samples were taken from two waterlogged peat swamp areas and

peat soil from an oil palm plantation that was originally peat swamp forest. In Pahang, all the soil samples were collected from peat swamp forest.

Soil samples from the top surface of the soil (10–15 cm) were taken and kept in polyethylene plastic bags. The soil samples were dried at $27\pm 1^\circ\text{C}$ for one week, ground into fine powder using a mortar and pestle, and sieved through a 2 mm strainer.

Three isolation methods, soil dilution plates, debris isolation, and direct isolation, were applied to recover *Fusarium* isolates from the peat soil samples. The three isolation methods were based on the methods described in The *Fusarium* Laboratory Manual (Leslie & Summerell 2006) for the isolation of *Fusarium* from soils.

Morphological Identification

Morphological identification was performed to sort the isolates into their respective groups before molecular identification was conducted. Isolates of *Fusarium* were tentatively identified based on the shapes of macroconidia, microconidia, chlamydospores, and conidiogenous cells observed on Carnation Leaf Agar, while pigmentation and colony appearance were observed on Potato Dextrose Agar. Species identification was based on the descriptions in The *Fusarium* Laboratory Manual (Leslie & Summerell 2006).

Molecular Identification

Molecular identification was performed using translation elongation factor-1 α (TEF-1 α) gene sequences. For phylogenetic analysis both TEF-1 α and β -tubulin gene sequences were used. The genomic DNA was extracted using DNeasy[®] Mini Plant Kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions.

The primers used to amplify TEF-1 α were EF1 (5'-ATG-GGT-AAG-GAG-GAC-AAG-AC-3') and EF2 (5'-GGA-AGT-ACC-AGT-GAT-CAT-GTT-3'), which were modified from O'Donnell *et al.* (1998). The primers used to amplify β -tubulin were BT1 (5'- AAC-ATG-CGT-GAG-ATT-GTA-AGT-3') and BT2 (5'-TAG-TGA-CCC-TTG-GCC-CAG-TTG-3'), as described by O'Donnell and Cigelnik (1997).

PCR reactions for both genes were carried out in 50 μL reaction mixtures containing 4 μL 5x PCR buffer, 4 mM MgCl_2 , 1.2 μL template DNA, 0.2 mM dNTPs mix (Promega, Madison, WI, USA), 1.25 U *Taq* DNA polymerase (Promega, Madison, WI, USA), and 0.7 mM of both forward and reverse primers.

PCR was performed in a DNA Engine[™] Peltier Thermal Cycler Model PTC-100 (MJ Research Inc., Watertown, MA, USA). PCR cycles for TEF-1 α were as follows: an initial denaturation at 94°C for 85 s, 35 cycles of denaturation at 95°C for 35 s, annealing at 59°C for 55 s, and extension at 72°C for 90 s, followed by a final extension at 72°C for 10 min. For PCR cycles for β -tubulin, the cycle started with an initial denaturation at 94°C for 1 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min.

Agarose gel (1%) electrophoresis was used to detect the PCR products. Electrophoresis was carried out using a Wealtec ELITE 300 (Meadowvale Way

Sparks, NV, USA) power supply and GES buffer tank with 1× Tris-borate-EDTA (TBE) buffer and was run for 90 min at 90 V and 400 mA. Ten mg/mL ethidium bromide (0.04 µL) was added to the gel to visualise PCR products. The size of the amplified bands was estimated using 1 kb and 100 bp DNA markers (Fermentas, Vilnius, Lithuania). The gel was viewed using a Bio-Rad Molecular Imager[®] Gel Doc[™] Xr system (Bio Rad, Hercules, CA, USA) with The Discovery Series[™] Quantity One[®] 1-D Analysis software Version 4.6.5 (Bio Rad, Hercules, CA, USA).

The PCR products were purified using a QIAquick[®] PCR Purification Kit (Qiagen, Germany) according to the manufacturers' instructions with some modifications to the volume of elution buffer used.

Phylogenetic Analysis

Sequences of TEF-1 α and β -tubulin were aligned using Clustal W included in Molecular Evolutionary Genetic Analysis (MEGA5) (Tamura *et al.* 2011). After pair-wise alignment, the sequences were compared with other sequences in the GenBank and Fusarium-ID databases using Basic Local Alignment Search Tool (BLAST). Two databases were used in this study for comparison and also to give accurate results for species identity based on the closest match of the BLAST search.

Phylogenetic analysis was conducted using a combined dataset of TEF-1 α and β -tubulin sequences. The phylogenetic tree was generated based on the Neighbour-Joining (NJ) method using MEGA5. *Fusarium denticulatum* was chosen as the out-group as this species was not closely related to any of the species identified in this study.

RESULTS

Isolation of *Fusarium* Isolates

A total of 70 isolates of *Fusarium* were discovered in the peat soil samples, of which 33 isolates were recovered from Sungai Beriah and Beriah Kecil, Perak, while 37 isolates were recovered from Sungai Bebar, Pahang (Table 1).

Based on morphological characteristics, five species were tentatively identified, namely *Fusarium oxysporum* (60%), *Fusarium solani* (23%), *Fusarium proliferatum* (14%), *Fusarium verticillioides* (1%), and *Fusarium semitectum* (1%). The morphological characteristics of the five *Fusarium* species are presented in Table 2.

A single band of 750 bp was detected using EF1 and EF2 primer pairs, indicating that TEF-1 α was successfully amplified from all 70 isolates recovered from the peat soil samples. A single band of 600 bp was detected using BT1 and BT2 primer pairs, indicating that β -tubulin was also successfully amplified from all 70 *Fusarium* isolates. All the sequences for both TEF-1 α and β -tubulin were deposited in GenBank and the accession numbers are indicated in supplementary files S1 and S2.

Based on the closest match of BLAST searches of TEF-1 α sequences, morphologically identified isolates demonstrated the same species identity with

isolates deposited in GenBank and Fusarium-ID databases with 93%–99% similarity (Table 3). Morphologically identified *F. semitectum* isolates showed 98% similarity to *F. incarnatum*, which is synonymous with *F. semitectum* (Leslie & Summerell 2006). Ten isolates morphologically identified as *F. proliferatum* showed 93%–99% similarity to *F. proliferatum* in both databases. One morphologically identified *F. verticillioides* (SBP48) isolate showed 99% similarity to *F. verticillioides*.

A total of 16 isolates morphologically identified as *F. solani* showed 93%–99% similarity to *F. solani*. All 42 isolates morphologically identified as *F. oxysporum* showed 94%–99% similarity to *F. oxysporum* in both databases.

Phylogenetic Analysis

A NJ tree constructed using a combination of TEF-1 α and β -tubulin sequences is shown in Figure 1. In the analysis, the sum of the branch length was 1.73 and a total of 1241 nucleotides were used. From the tree, the same isolates from the same species were clustered in the same clade. The outgroup, *F. denticulatum*, was clustered in a separate clade. The tree can be divided into two main clades, I and II, and main clade I can be further subdivided into sub-clades A, B, and C.

Sub-clade A can be divided into sub-sub-clades A1 and A2, in which sub-sub-clade A1 consists of *F. oxysporum* isolates with 100% bootstrap value, while sub-sub-clade A2 consists of *F. proliferatum* isolates with 100% bootstrap value. Sub-clades B and C comprise *F. verticillioides* and *F. semitectum*, respectively. Main clade II contained all *F. solani* isolates. Intraspecies variations were observed for isolates of *F. oxysporum*, *F. solani*, and *F. proliferatum*.

Table 1: Number of isolates recovered from the three sampled locations.

Locations	Total
Beriah Kecil and Sungai Beriah, Perak (oil palm plantation)	22
Sungai Beriah, Perak (water-logged)	11
Sungai Bebar, Pahang (peat swamp)	37
Total	70

Table 2: Morphological characteristics of *Fusarium* species isolated from peat soil.

<i>Fusarium</i> species	Microconidia	Macroconidia	Conidiogenous cell	Chlamydospore	Pigmentation
<i>F. oxysporum</i>	<ul style="list-style-type: none"> - Abundant in aerial mycelia - Oval shape - 0 septa 	<ul style="list-style-type: none"> - Abundant in sporodochia - Slender, falcate, and almost straight - 3–4 septa - Slightly hooked apical cell - Foot-shaped basal cell 	Short monophialides with false heads	Single or in pairs	Varied, from dark purple, dark red, white to violet, and white to creamy
<i>F. solarii</i>	<ul style="list-style-type: none"> - Abundant in aerial mycelia - Oval shape - 0 septa 	<ul style="list-style-type: none"> - Abundant in sporodochia - Stout with 3 septa - Curved and tapered end or blunt and rounded end apical cell - Foot-shaped or poorly developed basal cell 	Long monophialides with false head	Single or in pairs	Varied, from white to yellowish, white to creamy, and white to yellowish
<i>F. proliferatum</i>	<ul style="list-style-type: none"> - Varied, from oval, obovoid, or pyriform - 0 septa - Microconidia in chain (11–15 conidia) 	<ul style="list-style-type: none"> - Slender with 3–5 septa - Scarce - Curved and tapered apical cell - Poorly developed basal cell 	Monophialide and polyphialide with false head	Absent	White to light violet
<i>F. semitectum</i>	<ul style="list-style-type: none"> - Oval with 0 septa - Fusoid mesoconidia with 3–5 septa with appearance of rabbit ears 	<ul style="list-style-type: none"> - Slender and slightly curved with 3–5 septa - Curved and tapered apical cell - Foot-shaped basal cell 	Monophialide and polyphialide	Single or in pairs	Yellow to brownish
<i>F. verticillioides</i>	<ul style="list-style-type: none"> - Oval with 0 septa - Microconidia in long chain (more than 20 conidia) 	<ul style="list-style-type: none"> - Scarce - Slender with 3–5 septa - Tapered apical cell - Foot-shaped basal cell 	Monophialide	Absent	White to dark purple or light purple

Table 3: Percentage of sequence similarity, based on the TEF-1 α gene, of 70 *Fusarium* isolates from peat soil samples.

Code	TEF-1 α		Morphologically identified species
	GenBank (%)	Fusarium-ID (%)	
BKL24	<i>F. incarnatum</i> (98)	<i>Fusarium</i> sp.(93)	<i>F. semitectum</i>
SBP33	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i>
SBP34	<i>F. proliferatum</i> (98)	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i>
SBP35	<i>F. proliferatum</i> (98)	<i>F. proliferatum</i> (97)	<i>F. proliferatum</i>
SBP36	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i>
BKL22	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i>
BKL31	<i>F. proliferatum</i> (93)	<i>F. proliferatum</i> (93)	<i>F. proliferatum</i>
SBP41	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i>
SBP44	<i>F. proliferatum</i> (98)	<i>F. proliferatum</i> (98)	<i>F. proliferatum</i>
SBP47	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i> (99)	<i>F. proliferatum</i>
SBP40	<i>F. proliferatum</i> (97)	<i>F. proliferatum</i> (99)	<i>F. verticillioides</i>
SBP48	<i>F. verticillioides</i> (99)	<i>F. verticillioides</i> (99)	<i>F. verticillioides</i>
SBP39	<i>F. solani</i> (92)	<i>F. solani</i> (97)	<i>F. solani</i>
SBL25	<i>F. solani</i> (94)	<i>F. solani</i> (98)	<i>F. solani</i>
SBL23	<i>F. solani</i> (96)	<i>F. solani</i> (97)	<i>F. solani</i>
SBL21	<i>F. solani</i> (98)	<i>F. solani</i> (97)	<i>F. solani</i>
SBL24	<i>F. solani</i> (96)	<i>F. solani</i> (98)	<i>F. solani</i>
SBL26	<i>F. solani</i> (92)	<i>F. solani</i> (96)	<i>F. solani</i>
BKL12	<i>F. solani</i> (96)	<i>F. solani</i> (95)	<i>F. solani</i>
SBP11	<i>F. solani</i> (95)	<i>F. solani</i> (98)	<i>F. solani</i>
SBL10	<i>F. solani</i> (93)	<i>F. solani</i> (94)	<i>F. solani</i>
SBL11	<i>F. solani</i> (96)	<i>F. solani</i> (99)	<i>F. solani</i>
SBL12	<i>F. solani</i> (94)	<i>F. solani</i> (96)	<i>F. solani</i>
SBL13	<i>F. solani</i> (94)	<i>F. solani</i> (97)	<i>F. solani</i>
SBL14	<i>F. solani</i> (95)	<i>F. solani</i> (96)	<i>F. solani</i>
SBL15	<i>F. solani</i> (98)	<i>F. solani</i> (99)	<i>F. solani</i>
SBL16	<i>F. solani</i> (95)	<i>F. solani</i> (97)	<i>F. solani</i>
SBL17	<i>F. solani</i> (99)	<i>F. solani</i> (96)	<i>F. solani</i>
BKL11	<i>F. oxysporum</i> (94)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
BKL21	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
BKL26	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
BKL27	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
BKL28	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB401	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>

(continued on next page)

Table 3: (continued)

Code	TEF-1 α		Morphologically identified species
	GenBank (%)	Fusarium-ID (%)	
SB404	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB405	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB406	<i>F. oxysporum</i> (95)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB911	<i>F. oxysporum</i> (95)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB913	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB915	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB916	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB917	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB918	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB919	<i>F. oxysporum</i> (94)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB920	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB921	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB922	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB923	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB928	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB929	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB930	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB931	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB933	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB935	<i>F. oxysporum</i> (94)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB936	<i>F. oxysporum</i> (100)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB937	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB938	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB947	<i>F. oxysporum</i> (96)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB949	<i>F. oxysporum</i> (95)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB960	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB961	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i> (99)	<i>F. oxysporum</i>
SB971	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i> (98)	<i>F. oxysporum</i>
SB972	<i>F. oxysporum</i> (94)	<i>F. oxysporum</i> (97)	<i>F. oxysporum</i>
SB403	<i>F. oxysporum</i> (97)	<i>Fusarium</i> sp. (99)	<i>F. oxysporum</i>
SB914	<i>F. oxysporum</i> (99)	<i>Fusarium</i> sp. (99)	<i>F. oxysporum</i>
SB939	<i>F. oxysporum</i> (98)	<i>Fusarium</i> sp. (99)	<i>F. oxysporum</i>
SB941	<i>F. oxysporum</i> (96)	<i>Fusarium</i> sp. (99)	<i>F. oxysporum</i>
SB943	<i>F. oxysporum</i> (95)	<i>Fusarium</i> sp. (99)	<i>F. oxysporum</i>
SB944	<i>F. oxysporum</i> (96)	<i>Fusarium</i> sp. (99)	<i>F. oxysporum</i>
SB946	<i>F. oxysporum</i> (96)	<i>Fusarium</i> sp. (99)	<i>F. oxysporum</i>

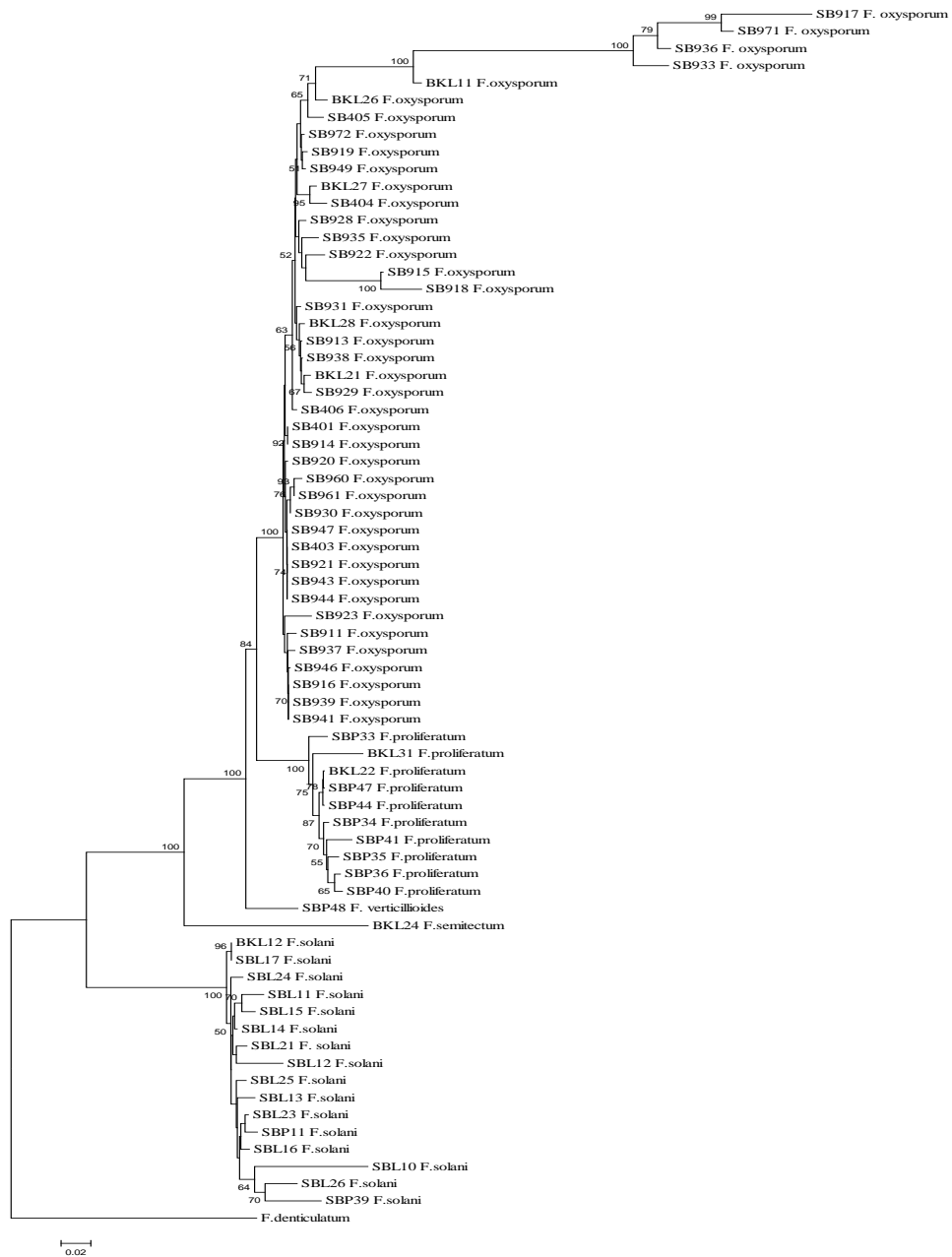


Figure 1: NJ tree of 70 isolates of *Fusarium* from peat soil samples based on TEF-1 α and β -tubulin sequences using the Jukes-Cantor method.

Notes: The bootstrap values higher than 50% are shown next to the branches. *F. denticulatum* is the out-group.

DISCUSSION

Five *Fusarium* species, namely *F. oxysporum*, *F. solani*, *F. proliferatum*, *F. verticillioides*, and *F. semitectum*, were isolated and identified from peat soil samples. Although several isolates showed a low percentage similarity in BLAST results, the information provides a clue regarding the species to which the isolates belong. The same species isolated from different hosts, substrates, and areas may show a few differences in nucleotide sequences compared with the deposited sequences in the databases (Geiser *et al.* 2004). Moreover, the threshold for species identity, such as the number of sequence differences needed to separate a species, was not set, and there is a high degree of potential variation of TEF-1 α alleles within a species (Geiser *et al.* 2004).

F. oxysporum is a soil inhabitant and one of the most widely distributed soil microorganisms (Gordon & Martyn 1997). *F. oxysporum* has been isolated from peat soils (Thormann & Rice 2007; Latiffah *et al.* 2010), from fen peat land (Stenton 1953), and from the rhizosphere and roots of smooth cordgrass (*Spartina alterniflora*) in the Dongtan wetlands in China (Luo *et al.* 2007). *F. oxysporum* has been reported in other extreme habitats, such as saline soil habitats (Mandeel 2006) and arid environments (Mandeel & Abbas 1994). Most *F. oxysporum* isolates were isolated from direct plating from the Sungai Bebar peat swamp forest. In the soil, *F. oxysporum* produces an abundance of chlamydospores and survives as propagules (Burgess 1981). When the peat soil was plated onto the media, the chlamydospores germinate and produced mycelium. This species has the ability to exist as a saprophyte in the soil and is able to degrade lignin (Sutherland *et al.* 1983; Rodrigues 1996).

From phylogenetic analysis, *F. oxysporum* isolates were clustered in several sub-clades, which indicates intraspecies variations. *F. oxysporum* is regarded as a species complex and has been reported to be a genetically heterogeneous morphospecies by O'Donnell and Cigelnik (1997) and Waalwijk *et al.* (1996). This species is also considered a monophyletic group and shows a diverse complex of evolutionary lineages (O'Donnell & Cigelnik 1997). The intraspecies variation may be due to its role as a soil inhabitant, as the species can be highly variable in growth characteristics on different substrates (Steinberg *et al.* 1997) and in ecological characteristics (Alabouvette *et al.* 1993).

F. solani was recovered from direct isolation and debris plating. This species is also commonly recovered from soil and plant debris and is one of the species that inhabits widely different types of soils, such as soils from sub-tropical and semi-arid conditions as well as grassland soils (Burgess & Summerell 1992), cultivated soils (Lim & Chew 1970; Latiffah *et al.* 2007), and sandy soils (Sarquis & Borba 1997). *F. solani* has also been isolated from peat soil samples in Malaysia (Latiffah *et al.* 2010) as well as from other extreme environments, such as saline soil habitats (Mandeel 2006) and arid habitats (Mandeel & Abbas 1994). *F. solani* produces chlamydospores, which will germinate and form mycelia during suitable conditions.

Similar to phylogenetic analysis of *F. oxysporum*, *F. solani* isolates were also clustered in the same main clade, with several sub-sub-clades that indicate intraspecies variations. *F. solani* also represents a species complex of 45

phylogenetic species that form the *F. solani* species complex (O'Donnell 2000). Within the *F. solani* species complex, worldwide isolates from soil and plant debris are usually grouped in one or two phylogenetic species, known as FSSC3 and FSSC4 (Zhang *et al.* 2006; O'Donnell *et al.* 2008). Intraspecies variations of *F. solani* have also been reported by Balmas *et al.* (2010), in which two phylogenetic species, FSSC5 and FSSC9, were identified among the 23 species of the *F. solani* species complex isolated from Sardinian soils.

F. proliferatum isolates were recovered using debris plating and dilution plating from peat soil samples in Sungai Beriah, Perak. They were also isolated from peat soils in Pondok Tanjung and Sungai Beriah (Latiffah *et al.* 2010) and from peat soil in Pelitanah in Sarawak (Omar *et al.* 2012). *F. proliferatum* has also been detected in non-agricultural soils in Australia (Sangalang *et al.* 1995; Summerell *et al.* 2003). Although *F. proliferatum* does not produce chlamydoconidia, the fungus survives due to its resistant hyphae and conidia that can also withstand extreme environmental conditions. In this current study, *F. proliferatum* isolates might be recovered from conidia as fungal colonies derived from soil dilution, a technique commonly used to recover isolates originating from spores or conidia (Griffin 1963). *F. proliferatum* produced microconidia and macroconidia, which could contribute to the occurrence of this species in peat soils.

F. proliferatum isolates also formed several sub-clades that demonstrated intraspecies variations. This species is a member of the *Fusarium fujikuroi* species complex and a common plant pathogen, occurring in various climatic regions. Genetic variation of *F. proliferatum* is commonly reported from isolates associated with crop plants. Genetic and phenotypic variations of *F. proliferatum* isolates from different hosts have been reported by Stepien *et al.* (2011). Amplified fragment length polymorphism (AFLP) analysis of *F. proliferatum* from onion using the TEF-1 α gene showed high genetic variation, where the isolates were clustered into two sub-clades with several sub-sub-clades (Galvan *et al.* 2008).

Although *F. semitectum* is common inhabitant of different types of soil, such as sandy soils (Sarquis & Borba 1997), grassland (Burgess *et al.* 1988), arctic (Kommedahl *et al.* 1988), and desert (Joffe *et al.* 1973), only one isolate was recovered from the peat soil samples from Beriah Kecil, Perak using the debris plating technique. *F. semitectum* has been isolated from peat soils in Pondok Tanjung and Sungai Beriah, Perak using direct plating and debris plating (Latiffah *et al.* 2010) in lower frequency compared to *F. oxysporum* and *F. solani*. *F. semitectum* isolates were clustered in main clade I, but formed a separate clade from other species. *F. semitectum* is synonymous with *F. incarnatum* and the species is in the *F. incarnatum-equiseti* species complex, with high genetic diversity (O'Donnell *et al.* 2009). Genetic variation in *F. semitectum* isolates has been reported by Masratul-Hawa *et al.* (2010) and Ingle and Rai (2011).

Only one isolate of *F. verticillioides* was isolated using the dilution plating technique. Based on a study by Liddell and Burgess (1985), isolates of *F. verticillioides* may live in plant residue on the soil surface. Moreover, *F. verticillioides* produced conidia that will germinate once suitable conditions are available. *F. verticillioides* has been isolated from some grassland soils in eastern

Australia (Summerell *et al.* 2011) and from mangrove soil in Malaysia (Latiffah *et al.* 2010). Similar with *F. proliferatum*, *F. verticillioides* does not produce chlamydospores, therefore the survival, reproduction, and dispersal of the species are dependent on microconidia and macroconidia (Glenn *et al.* 2004). The *F. verticillioides* isolate was clustered in the same main cluster with *F. oxysporum* and *F. proliferatum*, but formed a separate clade. *F. verticillioides* and *F. proliferatum* are members of *F. fujikuroi* species complex and thus these two species are closely related. *F. oxysporum* is grouped in section Elegans but formed a sister group with the *F. fujikuroi* species complex (Guadet *et al.* 1989; O'Donnell & Cigelnik 1997; O'Donnell *et al.* 2000). Genetic variation in *F. verticillioides* has been reported by Moretti *et al.* (2004) and Mirete *et al.* (2004).

The diversity of soil fungi is generally influenced by a large number of factors, such as soil pH, organic contents, and moisture (Rangaswami & Bagyaraj 1998). The acidic nature of the soil is influenced by heavy rainfall resulting in basic cations leaching out of the soil, leaving hydrogen and aluminium cations that make the soil acidic (Swier *et al.* 2011). Fungal growth favours acidic conditions. Other factors that can contribute to high fungal populations and affect the distribution of many *Fusarium* species in the soils include the availability of nutrients which promote fungal growth, such as nitrogen, phosphorus, and calcium, the level of organic carbon in the soil (Swier *et al.* 2011), and environmental factors, such as rainfall, temperature, soil type, and the type of vegetation present (Backhouse *et al.* 2001; Summerell *et al.* 2010). Thus, these factors may influence the occurrence and diversity of *Fusarium* isolates in peat soils.

In peat soils, *Fusarium* species are most likely saprophytes involved in the degradation of organic materials as well as nutrient cycling in the ecosystem. *Fusarium* species and other fungi also help in the carbon cycle and interact with plants through exchanging of organic and inorganic compounds (Tiwari *et al.* 2008). As saprophytes, *Fusarium* species act as decomposers and are regarded as group three degrading fungi, which degrade simple polymers (Deacon 1997). The simple polymers are mainly cellulose and pectin from plant debris. By secretion of extracellular enzymes such as cellulases, polyphenol oxidases, pectinases, and amylases, these polymers are degraded and nutrients are released into the ecosystems. Complex structural polymers consisting of mainly lignin and lignocellulose in the litter promote the growth of fungi, which in turn decompose the litter (Deacon 1997).

Chlamydospores are survival structures that assist *Fusarium* in survival in desiccated areas, high and low temperatures, and other harsh conditions (Leslie & Summerell 2006). Among the species isolated from the peat soil samples, *F. oxysporum*, *F. solani*, and *F. semitectum* produce chlamydospores. *Fusarium* species that do not form chlamydospores have adapted resistant conidia and hyphae for survival in extreme environments, or they remain dormant and are usually found in wetter areas (Burgess 1981). These structures will germinate and form in suitably extreme environmental conditions. The survival of *Fusarium* species using resistant hyphae and chlamydospores in soil and debris, such as *F. oxysporum*, has been reported by Vakalounakis and Chalkias (2004),

where it was found that *F. oxysporum* survived in the soil for more than 13 months.

Anamorphic Ascomycetes have been reported to be the largest group of fungi recovered from boreal peat lands (fens and bogs) in temperate regions (Thormann 2006; Thormann & Rice 2007). Among Ascomycetes, species of *Penicillium* are the main fungal taxon from boreal peat soil, followed by *Acremonium* spp., *Verticillium* spp., *Aspergillus* spp., *Trichoderma* spp., and *Fusarium* spp. (Thormann 2006). From bog and peat lands *Acremonium* spp. were the most common fungal taxon recorded, followed by *Aspergillus* spp., *Oidiodendron* spp., *Penicillium* spp., and *Trichoderma* spp. (Thormann 2006). Similar scenarios were also observed in tropical peat soils, where *Aspergillus* spp., *Penicillium* spp., and *Trichoderma* spp. were usually isolated. The occurrence of these fungal genera is not surprising as these taxa are regarded as generalists, heavy sporulators, and fast growing fungal groups (Thormann 2006).

Studies on *Fusarium* in non-agricultural soils, especially peat soil in peat swamp forest and waterlogged areas, provide insights on the survival and distribution of *Fusarium* species. Peat soil in these two areas could be a reservoir of plant pathogenic species, especially because well-known plant pathogens, such as *F. oxysporum*, *F. solani*, and *F. proliferatum*, were recovered. Moreover, plant pathogens have the potential to evolve in response to strong selection pressures imposed by agricultural ecosystems (Neumann *et al.* 2004) when the peat swamp areas are converted to agricultural use.

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S1: Accession numbers of TEF-1 α sequences of *Fusarium* species from peat soil.

Code	Accession number (TEF-1 α sequences)
BKL24 (<i>F. semitectum</i>)	KC120924
SBP33 (<i>F. proliferatum</i>)	KC120980
SBP34 (<i>F. proliferatum</i>)	KC120981
SBP35 (<i>F. proliferatum</i>)	KC120982
SBP36 (<i>F. proliferatum</i>)	KC120983
BKL22 (<i>F. proliferatum</i>)	KC120923
BKL31 (<i>F. proliferatum</i>)	KC120928
SBP41 (<i>F. proliferatum</i>)	KC120986
SBP44 (<i>F. proliferatum</i>)	KC120987
SBP47 (<i>F. proliferatum</i>)	KC120988
SBP40 (<i>F. proliferatum</i>)	KC120985
SBP48 (<i>F. verticillioides</i>)	KC120989
SBP39 (<i>F. solani</i>)	KC120984
SBL25 (<i>F. solani</i>)	KC120977
SBL23 (<i>F. solani</i>)	KC120975
SBL21 (<i>F. solani</i>)	KC120974
SBL24 (<i>F. solani</i>)	KC120976
SBL26 (<i>F. solani</i>)	KC120978
BKL12 (<i>F. solani</i>)	KC120921
SBP11 (<i>F. solani</i>)	KC120979
SBL10 (<i>F. solani</i>)	KC120966
SBL11 (<i>F. solani</i>)	KC120967
SBL12 (<i>F. solani</i>)	KC120968
SBL13 (<i>F. solani</i>)	KC120969
SBL14 (<i>F. solani</i>)	KC120970
SBL15 (<i>F. solani</i>)	KC120971
SBL16 (<i>F. solani</i>)	KC120972
SBL17 (<i>F. solani</i>)	KC120973
BKL11 (<i>F. oxysporum</i>)	KC120920
BKL21 (<i>F. oxysporum</i>)	KC120922
BKL26 (<i>F. oxysporum</i>)	KC120925
BKL27 (<i>F. oxysporum</i>)	KC120926
BKL28 (<i>F. oxysporum</i>)	KC120927
SB911 (<i>F. oxysporum</i>)	KC120934
SB913 (<i>F. oxysporum</i>)	KC120935
SB915 (<i>F. oxysporum</i>)	KC120937
SB916 (<i>F. oxysporum</i>)	KC120938
SB917 (<i>F. oxysporum</i>)	KC120939

(continued on next page)

S1: (continued)

Code	Accession number (TEF-1 α sequences)
SB919 (<i>F. oxysporum</i>)	KC120941
SB920 (<i>F. oxysporum</i>)	KC120942
SB921 (<i>F. oxysporum</i>)	KC120943
SB922 (<i>F. oxysporum</i>)	KC120944
SB923 (<i>F. oxysporum</i>)	KC120945
SB928 (<i>F. oxysporum</i>)	KC120946
SB929 (<i>F. oxysporum</i>)	KC120947
SB930 (<i>F. oxysporum</i>)	KC120948
SB931 (<i>F. oxysporum</i>)	KC120949
SB933 (<i>F. oxysporum</i>)	KC120950
SB935 (<i>F. oxysporum</i>)	KC120951
SB936 (<i>F. oxysporum</i>)	KC120952
SB937 (<i>F. oxysporum</i>)	KC120953
SB938 (<i>F. oxysporum</i>)	KC120954
SB947 (<i>F. oxysporum</i>)	KC120960
SB949 (<i>F. oxysporum</i>)	KC120961
SB960 (<i>F. oxysporum</i>)	KC120962
SB961 (<i>F. oxysporum</i>)	KC120963
SB971 (<i>F. oxysporum</i>)	KC120964
SB972 (<i>F. oxysporum</i>)	KC120965
SB403 (<i>F. oxysporum</i>)	KC120930
SB914 (<i>F. oxysporum</i>)	KC120936
SB939 (<i>F. oxysporum</i>)	KC120955
SB941 (<i>F. oxysporum</i>)	KC120956
SB943 (<i>F. oxysporum</i>)	KC120957
SB944 (<i>F. oxysporum</i>)	KC120958
SB946 (<i>F. oxysporum</i>)	KC120959

S2: Accession numbers of β -tubulin sequences of *Fusarium* species from peat soil.

Code	Accession number (β -tubulin sequences)
BKL24 (<i>F. semitectum</i>)	KC161303
SBP33 (<i>F. proliferatum</i>)	KC161319
SBP34 (<i>F. proliferatum</i>)	KC161320
SBP35 (<i>F. proliferatum</i>)	KC161326
SBP36 (<i>F. proliferatum</i>)	KC161327
BKL22 (<i>F. proliferatum</i>)	KC161323
BKL31 (<i>F. proliferatum</i>)	KC161325

(continued on next page)

S2: (continued)

Code	Accession number (β -tubulin sequences)
BKL31 (<i>F. proliferatum</i>)	KC161325
SBP41 (<i>F. proliferatum</i>)	KC161328
SBP44 (<i>F. proliferatum</i>)	KC161330
SBP47 (<i>F. proliferatum</i>)	KC161331
SBP40 (<i>F. proliferatum</i>)	KC161329
SBP48 (<i>F. verticillioides</i>)	KC161311
SBP39 (<i>F. solani</i>)	KC161321
SBL25 (<i>F. solani</i>)	KC161317
SBL23 (<i>F. solani</i>)	KC161308
SBL21 (<i>F. solani</i>)	KC161307
SBL24 (<i>F. solani</i>)	KC161309
SBL26 (<i>F. solani</i>)	KC161318
BKL12 (<i>F. solani</i>)	KC161302
SBP11 (<i>F. solani</i>)	KC161310
SBL10 (<i>F. solani</i>)	KC161304
SBL11 (<i>F. solani</i>)	KC161305
SBL12 (<i>F. solani</i>)	KC161306
SBL13 (<i>F. solani</i>)	KC161312
SBL14 (<i>F. solani</i>)	KC161313
SBL15 (<i>F. solani</i>)	KC161314
SBL16 (<i>F. solani</i>)	KC161315
SBL17 (<i>F. solani</i>)	KC161316
SB406 (<i>F. oxysporum</i>)	KC161339
SB911 (<i>F. oxysporum</i>)	KC161340
SB913 (<i>F. oxysporum</i>)	KC161341
SB915 (<i>F. oxysporum</i>)	KC161343
SB916 (<i>F. oxysporum</i>)	KC161344
SB918 (<i>F. oxysporum</i>)	KC161345
SB920 (<i>F. oxysporum</i>)	KC161347
SB921 (<i>F. oxysporum</i>)	KC161348
SB922 (<i>F. oxysporum</i>)	KC161349
SB923 (<i>F. oxysporum</i>)	KC161350
SB928 (<i>F. oxysporum</i>)	KC161351
SB929 (<i>F. oxysporum</i>)	KC161352
SB930 (<i>F. oxysporum</i>)	KC161353

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S2: (continued)

Code	Accession number (β -tubulin sequences)
SB931 (<i>F. oxysporum</i>)	KC161354
SB935 (<i>F. oxysporum</i>)	KC161355
SB937 (<i>F. oxysporum</i>)	KC161356
SB938 (<i>F. oxysporum</i>)	KC161357
SB947 (<i>F. oxysporum</i>)	KC161360
SB949 (<i>F. oxysporum</i>)	KC161361
SB960 (<i>F. oxysporum</i>)	KC161364
SB961 (<i>F. oxysporum</i>)	KC161366
SB972 (<i>F. oxysporum</i>)	KC161367
SB403 (<i>F. oxysporum</i>)	KC161336
SB914 (<i>F. oxysporum</i>)	KC161342
SB939 (<i>F. oxysporum</i>)	KC161365
SB941 (<i>F. oxysporum</i>)	KC161362
SB943 (<i>F. oxysporum</i>)	KC161363
SB944 (<i>F. oxysporum</i>)	KC161358
SB946 (<i>F. oxysporum</i>)	KC161359