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Molecular Phylogenetic Analysis of Indonesian *Fusarium* Isolates from Different Lifestyles, based on ITS Sequence Data

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Abstract

Fusarium species are pathogens, endophytes, and saprobes. Until recently, *Fusarium* species from Indonesia were mainly determined using morphology. In this study, molecular phylogenetic analysis of the *Fusarium* species complex in Indonesia, based on ITS rDNA sequence data, was carried out in order to analyze relationships among the *Fusarium* strains from different lifestyles. Strains isolated from plants, soil, litter, and other sources were used. Species belonging to six *Fusarium* species complexes, namely, *F. oxysporum*, *F. solani*, *F. decemcellulare*, *F. graminearum*, *F. fujikuroi*, and *F. tricinctum* were found. *Fusarium oxysporum* and *F. solani* are common species from Indonesia, found as saprobes, endophytes, and pathogens.

Key words – *Fusarium* diversity – identification – phylogenetic study

Introduction

Fusarium species are endophytes, saprobes, and pathogens to plants, animals, and humans. Plant pathogenic and endophytic fusaria have been reported associated with a wide range of plants (Summerbell & Schroers 2002, Schroers et al. 2004, 2009). Pathogenic *Fusarium* species have also been recorded infecting several animals such as insects and nematodes (O'Donnell et al. 2012), while saprobic species are distributed in soil, humus, and decayed wood worldwide (Schroers et al. 2009, Silvestro et al. 2013).

The genus *Fusarium* was described by Link in 1809, and sanctioned by Fries in 1821 (Aoki et al. 2014). Similar to other fungal genera, the basic identification of *Fusarium* until end of 1980's was morphological based (Aoki et al. 2014). Taxonomy and identification of species belonging to *Fusarium* have been shifting from morphology based to molecular based approach (Baayen et al. 2001, Geiser et al. 2004, O'Donnell et al. 2008, 2012). It was due to rapid development in fungal DNA extraction and the polymerase chain reaction (PCR) techniques which allowed rapid molecular identification of many fungal genera, including *Fusarium* (Oechsler et al. 2009). It was predicted that about 300 phylogenetically distinct species of

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Fusarium would exist, based on the molecular phylogenetic approach, however, most of them have yet to be described (Aoki et al. 2014).

Among many gene regions, the internal transcribed spacer (ITS) region is considered as a universal genetic marker for fungi, and it has the highest probability of successful identification for fungi using molecular approach (Schoch et al. 2012). Evaluation of 18S rDNA gene, ITS1, 5.8S rDNA, 28S rDNA, *β*-tubulin gene, and aminoadipate reductase gene (*lys2*) for inter-species identification of *Fusarium* showed that ratio of substitution rate of ITS1 regions is only lower than *lys2* gene and the percentages of inter-species nucleotide sequence homology ranged from 65– 100% (Watanabe et al. 2011). This indicated that ITS region is still valuable in identification majority of *Fusarium* species, however, the ITS region provides low resolution in revealing *Fusarium* members within species complexes. Until a new gene region is determined as a suitable marker for the identification of fungi, in particular *Fusarium*, utilization of sequence from the ITS region for diversity studies is preferred.

In the last five years, identification and diversity studies of *Fusarium* in Indonesia has moved from morphology to molecular approach (Pinaria et al. 2010, Jumjunidang et al. 2012, Nugroho et al. 2013, Nurbaya et al. 2014). However, information regarding species diversity of *Fusarium* from these studies has been confusing and possibly inaccurate due to the molecular identification approach used in most studies from Indonesia, which heavily relied on BLAST search results, without knowing the reliability of the data for *Fusarium* sequences available at GenBank (http://www.ncbi.nlm.nih.gov). It was noted that about 20% of the fungal sequence entries in GenBank may be incorrectly identified (Nilsson et al. 2006), therefore, screening for the reliability of sequence data retrieved from GenBank and other fungal databases for molecular phylogenetic analysis is very important. It was recommended to use *Fusarium*–ID (http://isolate.fusariumdb.org/index.php) and *Fusarium* MLST (http://www.cbs.knaw.nl/fusarium/) as sources of nucleotide sequences for molecular identification of *Fusarium* species (Aoki et al. 2014). In this study, diversity and phylogenetic relationship of *Fusarium* from Indonesia deposited at IPBCC (IPB Culture Collection) is elucidated using phylogenetic analysis based on nucleotide sequences generated from the ITS rDNA region.

Materials & Methods

Source of *Fusarium* isolates

The information regarding *Fusarium* isolates in this study is shown in Table 1. All strains were obtained from Bogor Agricultural University Culture Collection (IPBCC), Bogor, Indonesia.

DNA extraction, PCR amplification and sequencing

Isolates were re-cultured in potato dextrose broth (PDB) (Difco, USA) for 5 days at room temperature on a rotary shaker. Mycelium was harvested, air-dried, and freeze-dried in liquid nitrogen, and total genomic DNA was extracted according to the SDS method (Raeder & Broda 1985) with minor modification. In the PCR reaction, the primer set of ITS5 (forward) (5'–GGAAGTAAAAGTCGTAACAAGG–3') and ITS4 (reverse) (5'–TCCTCCGCTTATTGA-TAT–3') (White et al. 1990) was used to amplify the ITS region including 5.8S rDNA. The 30 µL PCR mixture contained 1.2 µL DNA template, 3 µL Dream Taq Buffer (including $MgCl_2$) (Thermo Scientific, USA), 3 µL 2 mM dNTP (Thermo Scientific, USA), 0.6 µL each 10 pmol primer (ITS5 and ITS4) (Thermo Scientific, USA), 0.75 µL Dream Taq polymerase (Thermo Scientific, USA), and 20.85 µL ddH_2O. PCR condition in the thermocycler was set as follows: pre-denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. All PCR results were further visualized using UV transilluminator after electrophoresis through a 1% agarose gel and ethidium bromide staining. A 1-kb molecular weight ladder (Thermo Scientific, USA) was included in each run. PCR products were sent to 1stBASE (Malaysia) for sequencing.

Table 1 List of 41 *Fusarium* strains studied, substrate or hosts, origin, lifestyle, and GenBank accession numbers.

Species Complex[#]	Species	Source	Substrate/Host	Origin	Lifestyle	GenBank accession number
FDSC	*F. decemcellulare*	IPBCC 13.1101	*Solanum melongena*	Bogor	Seed-borne	LC055813
	F. decemcellulare	IPBCC 14.1182	*S. melongena*	Bogor	Seed-borne	KR610403
	F. decemcellulare	IPBCC 14.1183	*S. melongena*	Bogor	Seed-borne	LC055814
	F. decemcellulare	IPBCC 14.1243	*S. melongena*	Bogor	Seed-borne	KR610404
	F. decemcellulare	IPBCC 14.1244	*S. melongena*	Bogor	Seed-borne	LC055820
FFSC	*Fusarium* sp.	IPBCC 08.580	Litter of *Shorea*	Tarakan	Saprobe	KR610399
	Fusarium sp.	IPBCC 10.657	Nest of insect	Pangandaran	Saprobe	KR610402
FGSC	*F. cerealis*	IPBCC 10.636	Soil	Bogor	Saprobe	LC055809
	Fusarium sp.	IPBCC 07.526	Agarwood of *Aquilaria*	West Papua	Endophyte	LC055793
	Fusarium sp.	IPBCC 11.881	Stem of *Uncaria gambier*	West Sumatra	Endophyte	LC055812
FOSC	*F. oxysporum*	IPBCC 07.328	Soil	Jambi	Saprobe	LC055790
	F. oxysporum	IPBCC 07.338	Soil	Jambi	Saprobe	LC055791
	F. oxysporum	IPBCC 07.540	Litter of *Shorea*	Central Kalimantan	Saprobe	LC055794
	F. oxysporum	IPBCC 08.561	Agarwood of *Aquilaria*	Central Bangka	Endophyte	LC055796
	F. oxysporum	IPBCC 08.562	Agarwood of *Aquilaria*	Central Bangka	Endophyte	LC055797
	F. oxysporum	IPBCC 08.565	Agarwood of *Aquilaria*	Central Bangka	Endophyte	LC055800
	F. oxysporum	IPBCC 08.568	Agarwood of *Aquilaria*	Sukabumi	Endophyte	LC055802
	F. oxysporum	IPBCC 08.582	Litter of *Shorea*	Tarakan	Saprobe	KR610400
	F. oxysporum	IPBCC 10.656	Nest of insect	Pangandaran	Saprobe	LC055810
	F. oxysporum	IPBCC 10.674	Nest of insect	Pangandaran	Saprobe	LC055811
	F. oxysporum	IPBCC 14.1236	Root of *Cinchona*	Bandung	Endopyhte	LC055815
	F. oxysporum	IPBCC 14.1237	Root of *Cinchona*	Bandung	Endophyte	LC055816
	F. oxysporum	IPBCC 14.1238	Root of *Cinchona*	Bandung	Endophyte	LC055817
	F. oxysporum	IPBCC 14.1239	Root of *Cinchona*	Bandung	Endophyte	LC055818
	F. oxysporum	IPBCC 14.1242	Root of *Cinchona*	Bandung	Endophyte	LC055819
	F. oxysporum	IPBCC 88.012	*Cucumis sativus*	–	Pathogen	LC055821
FSSC	*F. solani*	IPBCC 07.525	Agarwood of *Aquilaria*	Mataram	Endophyte	LC055792
	F. solani	IPBCC 08.560	Agarwood of *Aquilaria*	Central Bangka	Endophyte	LC055795
	F. solani	IPBCC 08.564	Agarwood of *Aquilaria*	Central Bangka	Endophyte	LC055799
	F. solani	IPBCC 08.566	Stem of *Aquilaria*	Central Bangka	Endophyte	LC055801
	F. solani	IPBCC 08.569	Stump of *Aquilaria*	Central Bangka	Endophyte	LC055803
	F. solani	IPBCC 08.570	Stump of *Aquilaria*	Central Bangka	Endophyte	LC055804
	F. solani	IPBCC 08.571	Stump of *Aquilaria*	Central Bangka	Endophyte	LC055805
	F. solani	IPBCC 08.574	Litter of bushes	Tarakan	Saprobe	KR610398
	F. solani	IPBCC 08.602	Litter of *Shorea*	Tarakan	Saprobe	LC055807
	Fusarium sp.	IPBCC 08.581	Litter of *Shorea*	Tarakan	Saprobe	LC055806
	Fusarium sp.	IPBCC 08.563	Agarwood of *Aquilaria*	Central Bangka	Endophyte	LC055798
	Fusarium sp.	IPBCC 08.612	Soil	Tarakan	Saprobe	KR610401
	Fusarium sp.	IPBCC 88.014*****	*S. tuberosum*	–	Pathogen	JX435207
FTSC	*Fusarium* sp.	IPBCC 10.635	Soil	Bogor	Saprobe	LC055808
	Fusarium sp.	IPBCC 88.017*****	–	–	–	LC055822

– : unknown substrate/host, origin, or lifestyle.

* : Strain was obtained from CBS (Centraalbureau voor Schimmelcultures), Utrecht, the Netherlands.

\# : FDSC (*F. decemcellulare* species complex), FFSC (*F. fujikuroi* species complex), FGSC (*F. graminearum* species complex), FOSC (*F. oxysporum* species complex), FSSC (*F. solani* species complex), FTSC (*F. tricinctum* species complex)

Phylogenetic analysis

DNA sequence generated from the ITS region was edited using ChromasPro version 1.7.7 (Technelysium, Australia). Multiple alignments were carried out in MEGA6 (Molecular Evolutionary Genetics Analysis Version 6.0) (Tamura et al. 2013) involving 41 nucleotide sequences from this study and sequences retrieved from GenBank (http://www.ncbi.nlm.nih.gov). The reliability of sequence data retrieved from GenBank was checked by comparing with data from *Fusarium*–ID (http://isolate.fusariumdb.org/index.php) and *Fusarium* MLST (http://www.cbs.knaw.nl/fusarium/). Sequence of *Hypocrea lixii* strain CBS 226.95 was used as outgroup (GenBank accession no. AF057606). The phylogenetic analysis was conducted using the maximum likelihood (ML) method in MEGA6. All parameter was set according to default program. The strength of the internal branches of the phylogenetic tree in ML analysis was tested with bootstrap (BS) analysis using 1000 replications. BS values of 50% or higher are shown.

Results

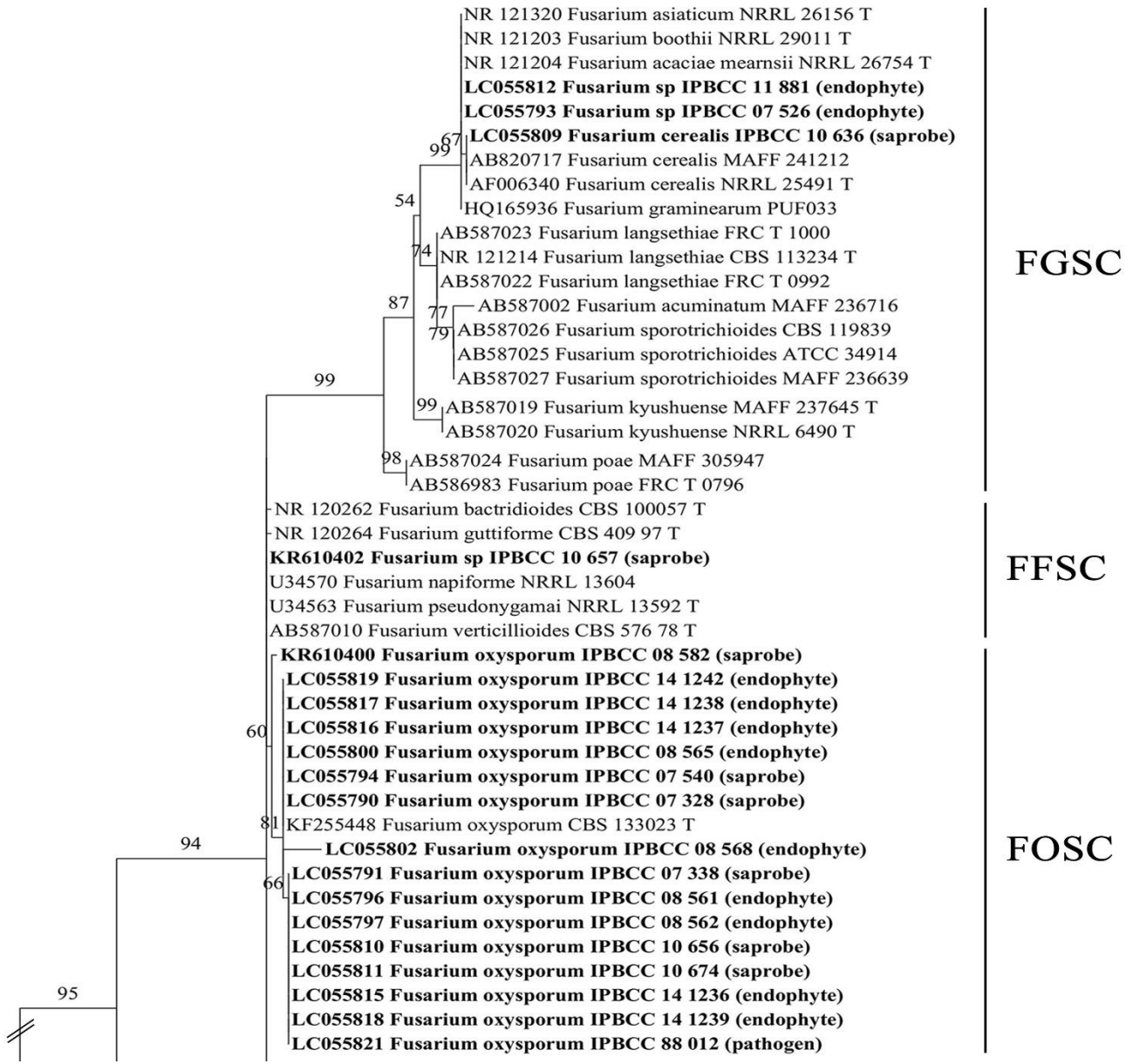
The alignment data matrix for ML analysis consisted of 89 sequences (including outgroup). Kimura-two parameter model with Gamma distribution was selected as the best DNA model of substitution in ML analysis for this dataset. The ITS tree showed that *Fusarium* sequences from this study belong to five distinct monophyletic clades represented species complexes, viz. *F. graminearum* species complex (FGSC) (BS=99%), *F. tricinctum* species complex (FTSC) (BS=99%), *F. decemcellulare* species complex (FDSC) (BS=100%), *F. solani* species complex (FSSC) (BS=98%), and *F. oxysporum* species complex (FOSC) (BS=60%). Another clade, FFSC was determined here as polyphyletic (Fig. 1).

ITS can be used for determination of species within FDSC, FGSC, FOSC, and FSSC, but not in FFSC and FTSC (Table 1). Five strains, viz. IPBCC 13.1101, IPBCC 14.1182, IPBCC 14.1183, IPBCC 14.1243, and IPBCC 14.1244 are nested in FDSC and determined as *F. decemcellulare*. One (IPBCC 10.636) of the three strains nested in FGSC is determined as *F. cerealis*. Within FOSC, sixteen *Fusarium* strains are determined as *F. oxysporum*, viz. IPBCC 07.328, IPBCC 07.338, IPBCC 07.540, IPBCC 08.561, IPBCC 08.562, IPBCC 08.565, IPBCC 08.568, IPBCC 08.582, IPBCC 10.656, IPBCC 10.674, IPBCC 14.1236, IPBCC 14.1237, IPBCC 14.1238, IPBCC 14.1239, IPBCC 14.1242, and IPBCC 88.012. Nine *Fusarium* strains within FSSC are determined as *F. solani*, viz. IPBCC 07.525, IPBCC 08.560, IPBCC 08.564, IPBCC 08.566, IPBCC 08.569, IPBCC 08.570, IPBCC 08.571, IPBCC 08.574, and IPBCC 08.602.

The tree also showed that *Fusarium* species with saprobe, endophyte, and pathogen lifestyles intermingled with each other in FGSC, FOSC, and FSSC clades (Fig. 1). Seed-borne *Fusarium* sequence from *Solanum melongena* nested in the same clade (FDSC), and additional sequences from seed-borne *Fusarium* are necessary to resolve whether this group of *Fusarium* is distinct from the *Fusarium* species with saprobe, endophyte, or pathogen lifestyle.

Discussion

Identifications of *Fusarium* in Indonesia using a molecular approach were reported by several authors in the last five years (Pinaria et al. 2010, Nugroho et al. 2013, Nurbaya et al. 2014), however, a phylogenetic study of the *Fusarium* species from Indonesia has not been carried out. This study showed the ITS rDNA sequence is still appropriate in determination of some *Fusarium* species, these include *F. cerealis*, *F. decemcellulare*, *F. oxysporum*, and *F. solani*. The ITS region offers a less complex tree, more efficient, low cost, and effective work (O'Donnell et al. 2008). Close related species such as *F. verticillioides* and *F. proliferatum* could also be differentiated based on sequence from the ITS region (Visentin et al. 2009).


NR 121320 Fusarium asiaticum NRRL 26156 T
NR 121203 Fusarium boothii NRRL 29011 T
NR 121204 Fusarium acaciae mearnsii NRRL 26754 T
LC055812 Fusarium sp IPBCC 11 881 (endophyte)
LC055793 Fusarium sp IPBCC 07 526 (endophyte)
LC055809 Fusarium cerealis IPBCC 10 636 (saprobe)
AB820717 Fusarium cerealis MAFF 241212
AF006340 Fusarium cerealis NRRL 25491 T
HQ165936 Fusarium graminearum PUF033
AB587023 Fusarium langsethiae FRC T 1000
NR 121214 Fusarium langsethiae CBS 113234 T
AB587022 Fusarium langsethiae FRC T 0992
AB587002 Fusarium acuminatum MAFF 236716
AB587026 Fusarium sporotrichioides CBS 119839
AB587025 Fusarium sporotrichioides ATCC 34914
AB587027 Fusarium sporotrichioides MAFF 236639
AB587019 Fusarium kyushuense MAFF 237645 T
AB587020 Fusarium kyushuense NRRL 6490 T
AB587024 Fusarium poae MAFF 305947
AB586983 Fusarium poae FRC T 0796
NR 120262 Fusarium bactridioides CBS 100057 T
NR 120264 Fusarium guttiforme CBS 409 97 T
KR610402 Fusarium sp IPBCC 10 657 (saprobe)
U34570 Fusarium napiforme NRRL 13604
U34563 Fusarium pseudonygamai NRRL 13592 T
AB587010 Fusarium verticillioides CBS 576 78 T
KR610400 Fusarium oxysporum IPBCC 08 582 (saprobe)
LC055819 Fusarium oxysporum IPBCC 14 1242 (endophyte)
LC055817 Fusarium oxysporum IPBCC 14 1238 (endophyte)
LC055816 Fusarium oxysporum IPBCC 14 1237 (endophyte)
LC055800 Fusarium oxysporum IPBCC 08 565 (endophyte)
LC055794 Fusarium oxysporum IPBCC 07 540 (saprobe)
LC055790 Fusarium oxysporum IPBCC 07 328 (saprobe)
KF255448 Fusarium oxysporum CBS 133023 T
LC055802 Fusarium oxysporum IPBCC 08 568 (endophyte)
LC055791 Fusarium oxysporum IPBCC 07 338 (saprobe)
LC055796 Fusarium oxysporum IPBCC 08 561 (endophyte)
LC055797 Fusarium oxysporum IPBCC 08 562 (endophyte)
LC055810 Fusarium oxysporum IPBCC 10 656 (saprobe)
LC055811 Fusarium oxysporum IPBCC 10 674 (saprobe)
LC055815 Fusarium oxysporum IPBCC 14 1236 (endophyte)
LC055818 Fusarium oxysporum IPBCC 14 1239 (endophyte)
LC055821 Fusarium oxysporum IPBCC 88 012 (pathogen)
FGSC
FFSC
FOSC

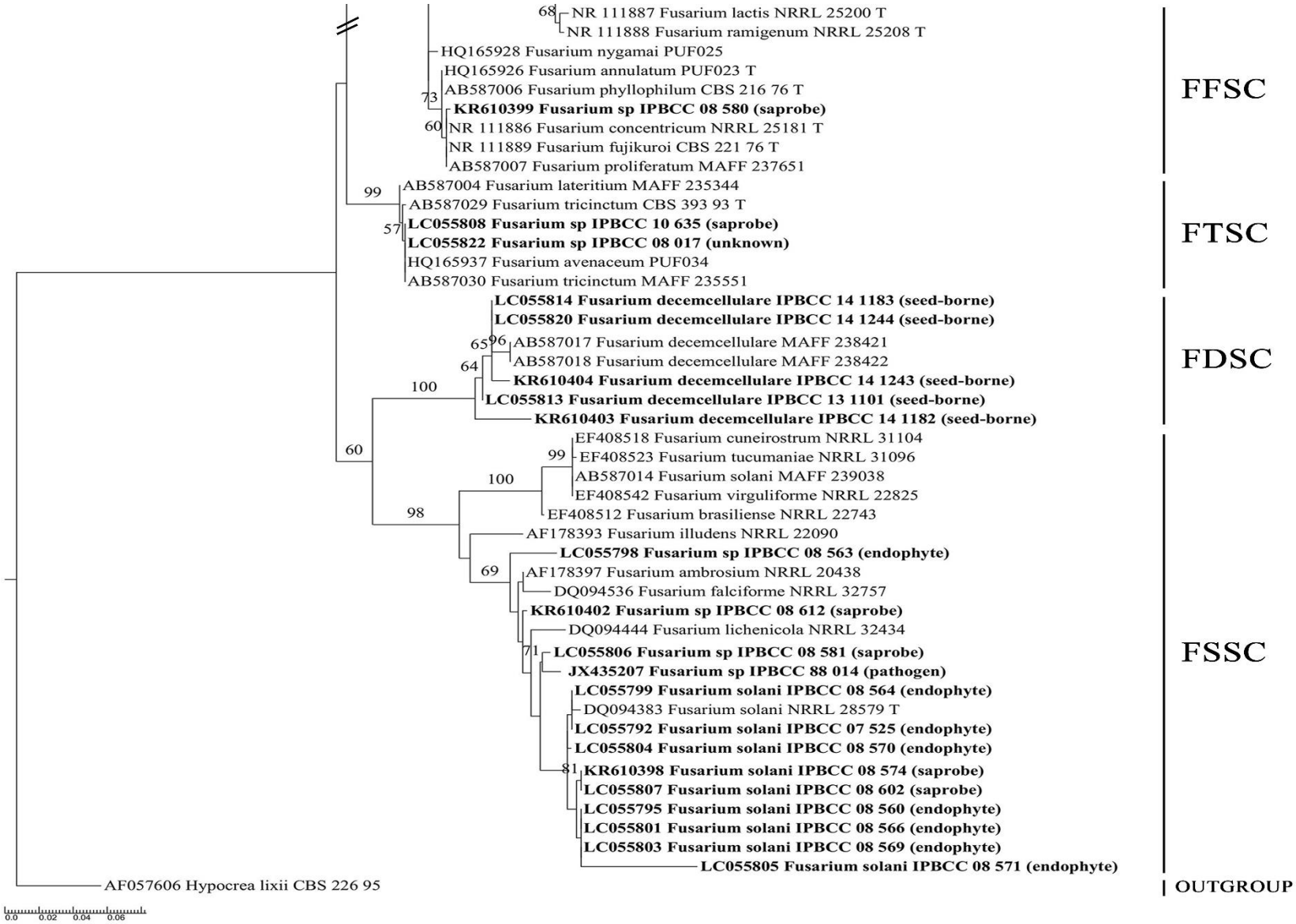


Fig. 1 – Maximum Likelihood phylogenetic tree for *Fusarium* species from Indonesia based on complete ITS ribosomal DNA sequence. Number over branches represents bootstrap support value from 1000 replicates (≥50%). Bold characters represent strain used in this study. FDSC (*F. decemcellulare* species complex), FFSC (*F. fujikuroi* species complex), FGSC (*F. graminearum* species complex), FOSC (*F. oxysporum* species complex), FSSC (*F. solani* species complex), FTSC (*F. tricinctum* species complex).

However, this region is not sufficient to discriminate other members of *F. fujikuroi* complex such as *F. verticillioides* with *F. subglutinans* (O'Donnell & Cigelnik 1997), or *F. fujikuroi* with *F. proliferatum* (Waalwijk et al. 1996). Least informative or low nucleotide sequence variation of the ITS region to clearly determine several species complex was also previously stated (Oechsler et al. 2009, Wang et al. 2011). This information suggested that researchers have to be careful during interpretation of the phylogenetic tree generated from ITS sequences (Cheng et al. 2008, O'Donnell et al. 2008).

This study showed that *Fusarium* sequences from different hosts or sources nested in the same species complex as shown in FOSC and FSSC clades. A wide host range in FOSC (Laurence et al. 2014) and FSSC (Suga et al. 2000) has been noted. The FOSC and FSSC are complex species which contain multiple morphologically cryptic species, and nucleotide sequence variation from the ITS region is not sufficient in differentiating the cryptic species within these species complexes. Genealogical Concordance Phylogenetic Species Recognition (GCPSR) concept using multigene approach from coding and non-coding region is considered best for the discrimination of species within FOSC and FSSC species complexes (Short et al. 2013, Laurence et al. 2014).

Species complexes in *Fusarium* may have different lifestyle. For example, the FOSC clade (n=16) composed of endophytic sequences from *Aquilaria* and *Cinchona* tree (n=9), pathogen of *C. sativus* (n=1), saprobes from plant litter, insect nest, and soil (n=6). The FSSC clade contains less diverse sources such as endophytes of *Aquilaria* (n=8), pathogen from unknown source (n=1), and saprobes in litter and soil (n=4). The FGSC clade (n=3) was also composed of endophytic (n=2) and saprobic (n=1). In the FTSC clade, *Fusarium* strains (n=2) were saprobe and unknown source. Further, a single species of *Fusarium* may have different lifestyle as endophytes, pathogens, and saprobes. The spectrum of the *Fusarium* species complex may also differ in several respects. FOSC which is the most common species complex, has been reported to be saprobe, endophyte, and pathogen. Member of FOSC and FSSC have not been reported to be seed-borne, while all member of FDSC were seed-borne on *S. melongena*. Although most fusaria included in the present study have not been reported to cause infections in human and animal, some human pathogenic fusaria have been reported (Summerbell & Schroers 2002, O'Donnell et al. 2008, Oechsler et al. 2009, Schroers et al. 2009, Wang et al. 2011). *Fusarium coccophilum*, which was recorded by O'Donnell et al. (2012) to associate with scale insect in Indonesia, has not been found in the present study.

Studies on molecular phylogenetic analysis of *Fusarium* species from several genetic loci have been done by many investigators, viz. *F. redolens* and *F. hostae* (mtSSU rDNA and EF–1α) (Baayen et al. 2001), *F. oxysporum* and *F. commune* (ITS, mtSSU, and EF–1α) (Stewart et al. 2006), FSSC and FTSC (ITS, LSU, EF–1α, and RPB2) (O'Donnell et al. 2008), FIESC and FCSC (EF–1α, RPB2, ITS+LSU, CAM) (O'Donnell et al. 2009), FDSC (β–tub, ITS, LSU, and EF-1α) (Schroers et al. 2009), and FTSC, FIESC, FSAMSC, FOSC, GFSC, FSSC, FDSC (ITS, LSU, IGS, mtSSU, EF–1α, CAM, and RPB2) (Wang et al. 2011). Among the seven loci studied by Wang et al. (2011), the ITS, mtSSU, and LSU rRNA genes were least informative. For the identification of human pathogenic fusaria to species level, gene regions of EF–1α, RPB1, and RPB2 were suggested (O'Donnell et al. 2010). Recently, 22 species complexes were recognized for agriculture and medical Fusaria by using RPB1 and RPB2 (O'Donnell et al. 2013). To identify to at least the species complex level, EF–1α was very capable to assign the strain due to high sequence similarity for the known *Fusarium* species in the *Fusarium*–ID database. Wang et al. (2011) suggested the IGS was the most informative locus to differentiate *Fusarium* strains, while RPB2 was less discriminatory than the IGS and the EF–1α gene. Another gene region such as aminoadipate reductase gene (*lys2*) was also proposed as a suitable marker for the identification of *Fusarium* members (Watanabe et al. 2011, Watanabe 2013). In fact, each of proposed genes from *Fusarium* genome has possibly unique evolutionary history (Watanabe et al. 2011).

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References

Aoki T, O'Donnell K, Geiser DM. 2014 – Systematic of key phytopathogenic *Fusarium* species: current status and future challenges. Journal of General Plant Pathology 80, 189–201. Doi: 10.1007/s10327-014-0509-3.

Baayen RP, O'Donnell K, Breeuwsma S, Geiser DM, Waalwijk C. 2001 – Molecular relationships of fungi within the *Fusarium redolens–F. hostae* clade. Phytopathology 91, 1031–1044. Doi: 10.1094/PHYTO.2001.91.11.1037.

Cheng Z, Tang W, Su Z, Cai Y, Sun S, Chen Q, Wang F, Lin Y, She Z, Vrijmoed LLP. 2008 – Identification of mangrove endophytic fungus 1403 (*Fusarium proliferatum*) based on morphological and molecular evidence. Journal of Forestry Research 19, 219–224. Doi: 10.1007/s11676-008-0030-7.

Geiser DM, Jime´nez-Gasco MM, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, Zhang N, Kuldau GA, O'Donnell K. 2004 – FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium.* European Journal of Plant Pathology 110, 473–479. Doi: 10.1023/B:EJPP.0000032386.75915.a0.

Jumjunidang, Riska, Soemargono. 2012 – Identification and distribution of *Fusarium oxysporum* f. sp. *cubense* isolates through analysis of vegetative compatibility group in Lampung Province, Indonesia. ARPN Journal of Agricultural and Biological Science 7, 279–284.

Laurence HM, Summerell BA, Burgess LW, Liew ECY. 2014 – Genealogical concordance phylogenetic species recognition in the *Fusarium oxysporum* species complex. Fungal Biology 118, 374–384. Doi: 10.1016/j.funbio.2014.02.002.

Nilsson RH, Ryberg M, Kristiansson E, Larsson KH, Kõljalg U. 2006 – Taxonomic reliability of DNA sequences in public sequences databases: a fungal perspective. PLoS ONE 1, e59. Doi: 10.1371/journal.pone.0000059.

Nugroho PA, Setyabudi FMCS, Saleh B, Rahayu ES. 2013 – Fumonisin–producing *Fusarium* from maize grains in Tretep, Indonesia. Journal of Food Science and Engineering 3, 534–540.

Nurbaya, Kuswinanti T, Rosmana A, Baharuddin, Millang S. 2014 – Growth rate and identification of *Fusarium* spp. associated with *Aquillaria* spp. from Nunukan regency, North Kalimantan. International Journal of Current Research and Academic Review 2, 33–40.

O'Donnell K, Cigelnik E. 1997 – Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are non-orthologous. Molecular Phylogenetics and Evolution 1, 1–14. Doi: 10.1006/mpev.1996.0376.

O'Donnell K, Sutton DA, Fothergill A, McCarthy D, Rinaldi MG, Brandt ME, Zhang N, Geiser DM. 2008 – Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. Journal of Clinical Microbiology 46, 2477–2490. Doi: 10.1128/JCM.02371-07.

O'Donnell K, Sutton DA, Rinaldi MG, Gueidan C, Crous PW, Geiser DM. 2009 – Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum–F. equiseti* and *F. chlamydosporum* species complexes within the United States. Journal of Clinical Microbiology 47, 3851–3861. Doi:10.1128/JCM.01616-09.

O'Donnell K, Sutton DA, Rinaldi MG, Sarver BAJ, Balajee SA, Schroers HJ, Summerbell RC, Robert VA, Crous PW, Zhang N, Aoki T, Jung K, Park J, Lee YH, Kang S, Park B, Geiser DM. 2010 – Internet–accessible DNA sequence database for identifying fusaria from human and animal infections. Journal of Clinical Microbiology 48, 3708–3718. Doi: 10.1128/JCM.00989-10.

O'Donnell K, Humber RA, Geiser DM, Kang S, Crous PW, Johnston PR, Aoki T, Rooney AP, Rehner SA. 2012 – Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via FUSARIUM-ID and *Fusarium* MLST. Mycologia 104, 427–445. Doi: 10.3852/11-179.

O'Donnell K, Rooney AP, Proctor RH, Brown DW, McCormick SP, Ward TJ, Frandsen RJN, Lysøe E, Rehner SA, Aoki T, Robert VARG, Crous PW, Groenewald JZ, Kang S, Geiser DM. 2013 – Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genetics and Biology 52, 20–31. http://dx.doi.org/10.1016/j.fgb.2012.12.004

Oechsler RA, Feilmeier MR, Ledee DR, Miller D, Diaz MR, Fini ME, Fell JW, Alfonso EC. 2009 – Utility of molecular sequence analysis of the ITS rRNA region for identification of *Fusarium* spp. from ocular sources. Investigative Ophthalmology & Visual Science 50, 2230–2236. Doi: 10.1167/iovs.08-2757.

Pinaria AG, Liew ECY, Burgess LW. 2010 – *Fusarium* species associated with vanilla stem rot in Indonesia. Australasian Plant Pathology 39, 176–183. Doi: 10.1071/AP09079.

Raeder U, Broda P. 1985 – Rapid preparation of DNA from filamentous fungi. Letters in Applied Microbiology 1, 17–20. Doi: 10.1111/j.1472-765X.1985.tb01479.x.

Schoch CL, Seifert K, Huhndorf S et al. 2012 – Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America 109, 6241–6246. Doi: 10.1073/pnas.1117018109.

Schroers HJ, Baayen RP, Meffert JP, Gruyter J, Hooftman M, O'Donnell K. 2004 – *Fusarium foetens*, a new species pathogenic to begonia elatior hybrids (*Begonia* × *hiemalis*) and the sister taxon of the *Fusarium oxysporum* species complex. Mycologia 96, 393–406. Doi: 10.2307/3762070.

Schroers HJ, O'Donnell KO, Lamprecht SC, Kammeyer PL, Johnson S, Sutton DA, Rinaldi MG, Geiser DM, Summerbell RC. 2009 – Taxonomy and phylogeny of the *Fusarium dimerum* species group. Mycologia 101, 44–70. Doi: 10.3852/08-002.

Short DPG, O'Donnell K, Thrane U, Nielsen KF, Zhang N, Juba JH, Geiser DM. 2013 – Phylogenetic relationships among members of the *Fusarium solani* species complex in human infections and the descriptions of *F. keratoplasticum* sp. nov. and *F. petroliphilum* stat. nov. Fungal Genetics and Biology 53, 59–70. Doi: 10.1016/j.fgb.2013.01.004.

Silvestro LB, Stenglein SA, Forjan H, Dinolfo MI, Arambarri AM, Manso L, Moreno MV. 2013 – Occurrence and distribution of soil *Fusarium* species under wheat crop in zero tillage. Spanish Journal Agricultural Research 11, 72–79. Doi: 10.5424/sjar/2013111-3081.

Stewart JE, Kim MS, James RL, Dumroese RK, Klopfenstein NB. 2006 – Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* isolates from a conifer nursery. Phytopathology 96, 1124–1133. Doi: 10.1094/PHYTO-96-1124.

Suga H, Hasegawa T, Mitsui H, Kageyama K, Hyakumachi M. 2000 – Phylogenetic analysis of the phytopathogenic fungus *Fusarium solani* based on the rDNA–ITS region. Mycological Research 104, 1175–1183. Doi: 10.1017/S0953756200002719.

Summerbell RC, Schroers HJ. 2002 – Analysis of phylogenetic relationship of *Cylindrocarpon lichenicola* and *Acremonium falciforme* to the *Fusarium solani* species complex and a review of similarities in the spectrum of opportunistic infections caused by these fungi. Journal of Clinical Microbiology 40, 2866–2875. Doi: 10.1128/JCM.40.8.2866-2875.2002.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013 – MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30, 2725–2729. Doi: 10.1093/molbev/mst197.

Visentin I, Tamietti G, Valentino D, Portis E, Karlovsky P, Moretti A, Cardinale F. 2009 – The ITS region as a taxonomic discriminator between *Fusarium verticillioides* and *Fusarium proliferatum*. Mycological Research 113, 1137–1145. Doi: 10.1016/j.mycres.2009.07.011.

Waalwijk C, De Koning JRA, Baayen RP, Bayen RP, Gams W. 1996 – Discordant grouping of *Fusarium* spp. from sections *Elegans*, *Liseola*, *Dlaminia* based on ribosomal ITS1 and ITS2 sequences. Mycologia 88, 361–368. Doi: 10.2307/3760877.

Wang H, Xiao M, Kong F, Chen S, Dou HT, Sorrell T, Li RY, Xu YC. 2011 – Accurate and practical identification of 20 *Fusarium* species by seven-locus sequence analysis and reverse

line blot hybridization, and an in vitro antifungal susceptibility study. Journal of Clinical Microbiology 49, 1890–1898. Doi: 10.1128/JCM.02415-10.

Watanabe M. 2013 – Molecular phylogeny and identification of *Fusarium* species based on nucleotide sequences. Mycotoxins 63, 133–142. Doi: 10.2520/myco.63.133.

Watanabe M, Yonezawa T, Lee K, Kumagai S, Sugita–Konishi Y, Goto K, Hara–Kudo Y. 2011 – Evaluation of genetic markers for identifying isolates of the species of the genus *Fusarium*. Journal of the Science of Food and Agriculture 91, 2500–2504. Doi: 10.1002/jsfa.4507.

White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR: A Guide to Methods and Applications. Edited by: Innis MA, Gelfand DH, Sninsky JJ, White TJ, Academic Press, New York, US, 315–322 pp.