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Fusarium Species Isolated from Mangrove Soil in Kampung Pantai Acheh, Balik Pulau, Pulau Pinang, Malaysia

Latiffah Zakaria*, Mah Kok Foong, Heng Mei Hsuan, Maziah Zakaria and Baharuddin Salleh

School of Biological Sciences, Universiti Sains Malaysia,
11800 USM, Pulau Pinang, Malaysia

Abstrak: Sebanyak 33 pencilan *Fusarium* telah dipencilkan daripada sampel tanah di satu kawasan hutan paya bakau di Kampung Pantai Acheh, Balik Pulau, Pulau Pinang. Kesemua pencilan tersebut diperolehi menggunakan kaedah pencairan tanah, pemencilan terus dan pemencilan sisabaki tumbuhan. Kaedah pemencilan sisabaki tumbuhan menghasilkan pencilan *Fusarium* yang paling banyak iaitu 22 pencilan. Berdasarkan ciri-ciri morfologi, tiga spesies *Fusarium* dapat dikenalpasti iaitu *F. solani*, *F. oxysporum* dan *F. verticillioides*. *F. solani* (91%) merupakan spesies yang paling kerap diperolehi daripada sampel tanah hutan paya bakau diikuti dengan *F. oxysporum* (6%) and *F. verticillioides* (3%).

Kata kunci: *Fusarium*, Paya Bakau, Tanah

Abstract: A total of 33 isolates of *Fusarium* sp. were isolated from soil samples collected from a mangrove forest in an area in Kampung Pantai Acheh, Balik Pulau, Pulau Pinang, Malaysia. The isolates were isolated using soil dilution, direct isolation and debris isolation techniques. The debris isolation technique yielded the most isolates, with a total of 22 *Fusarium* isolates. Based on identification using morphological characteristics, three *Fusarium* species were identified: *F. solani*, *F. oxysporum* and *F. verticillioides*. *F. solani* (91%) was the most common species recovered from the mangrove soil samples, followed by *F. oxysporum* (6%) and *F. verticillioides* (3%).

Keywords: *Fusarium*, Mangrove, Soil

INTRODUCTION

Mangrove forests are located at the interface between land and sea, a unique and extreme environment. Mangrove communities are comprised of many different types of flora and fauna and can withstand high levels of salinity and temperature, extreme tides, and muddy anaerobic conditions (Kathiresan & Bingham 2001).

The soils in mangrove communities are muddy or sandy with loose sediments. They contain submerged mangrove roots, trunks and branches. These conditions attract rich communities of fungi and bacteria (Kathiresan & Bingham 2001). Among the microorganisms, the fungal community is the principal degrader of plant debris, especially during the early phases of decomposition. Therefore, fungi play an important role in the transformation and cycling of nutrients in the ecosystem.

*Corresponding author: Lfah@usm.my

The fungus *Fusarium* has previously been recovered from mangrove soils and debris (Rai *et al*. 1966; Khallil *et al*. 1991; Mehdi & Saifullah 2000; Tariq *et al*. 2008), and its occurrence suggests it can withstand the extreme environment of the mangrove ecosystem. However, recovery of *Fusarium* from mangroves is still relatively rare, warranting further research in Malaysia. This preliminary study was conducted to survey the occurrence of *Fusarium* species in a mangrove area in Kampung Pantai Acheh, Balik Pulau, Pulau Pinang.

MATERIALS AND METHODS

Soil Sampling

Five soil samples were collected from an area of mangrove forest in Kampung Pantai Acheh, Balik Pulau, Pulau Pinang, and were systematically numbered, with M referring to a particular mangrove tree. The vegetation in the area consisted mainly of *Avicennia* species. The soil samples were taken from a depth of 1–10 cm and then kept in plastic bags until drying was performed immediately after sampling in the laboratory.

The soil samples were air-dried at room temperature (27±1°C) for seven days and then ground using a mortar and pestle. Ground soil samples were sieved with a 0.5 mm sieve to remove larger particle such as stones and plant debris in order to obtain a consistent soil particle size for isolation using the soil dilution technique (below). Sieved soils and debris were then stored separately in paper bags and kept at 4°C .

Isolation and Identification of *Fusarium* Species

Three methods, namely soil dilution agar plates, debris isolation and direct isolation, were used to isolate species of *Fusarium* from mangrove soil. These techniques were based on the methods described in The *Fusarium* Laboratory Manual (Leslie & Summerell 2006). For the soil dilution technique, the number of colony-forming units (CFUs) on the media was recorded.

Isolates of *Fusarium* were identified using primary and secondary morphological characteristics according to the Nelson *et al*. (1983) identification classification manual and The *Fusarium* Laboratory Manual (Leslie & Summerell 2006). Peptone chloronitrobenzene (PCNB) was added to the media used for isolation, and carnation leaf-piece agar (CLA) and potato dextrose agar (PDA) were used for identification. Isolates from the section Liseola were reconfirmed by sequencing of the α-translation elongation factor gene (Geiser *et al.* 2004). The section Liseola consists of asexual states or anamorphs of *Gibberella fujikuroi* species complex. *Giberrella* species are sexual states or teleomorphs of *Fusarium* species.

Soil Analysis

Soil samples from the mangrove swamp were analysed for texture, pH, and moisture content. Soil texture was classified by the method of Thein (1979) according to the soil's general appearance and 'feel' when squeezed and fashioned into a ribbon. The separate soil texture compositions are defined based on the USDA Texture Triangle.

Soil pH was measured by weighing 30 g of the soil sample and put in a 100 ml beaker. Seventy five ml of distilled water was added and mixed well by stirring to obtain soil slurry. The mixture was then incubated at room temperature (27±1°C) for 24 h to allow the pH of the soil slurry to stabilise. An average of three pH readings was taken and recorded after the 24 h incubation period (Head 1980).

RESULTS

A total of 33 isolates of *Fusarium* were isolated from the mangrove soil samples. The *Fusarium* species isolated and the soil texture, pH and moisture content are shown in Table 1.

Based on morphological characteristics (Table 2), three *Fusarium* species were identified: *F. solani* (91%), *F. oxysporum* (6%) and *F. verticillioides* (3%). Because *F. verticillioides* is a member of the *G. fujikuroi* species complex, the isolate was reconfirmed by sequencing the α-translation elongation gene and performing a BLAST comparison against GenBank.

Debris plating yielded the most isolates of *Fusarium*. Nineteen isolates of *F. solani,* two isolates of *F. oxysporum* and one isolate of *F. verticilioides* were identified. Using the soil dilution technique, only two isolates of *F. solani* were obtained from two soil samples (M1 and M5). Nine isolates of *F. solani* were recovered via direct isolation, while the M4 soil sample yielded no *Fusarium* isolates (Table 1).

The texture of the mangrove soil samples was a silty clay loam. It was gritty, but could be moulded into ribbons without fractures. The 5 soil samples were about 27%–40% clay, and ribbons up to 7.5 cm were resistant to shear. The soil samples were smooth to the touch, as the soil particles were a fine powder.

The mangrove soil samples ranged in pH from 7.23 to 7.52, which was slightly alkaline. The moisture content of the mangrove soil samples was 5%–6% (Table 1).

DISCUSSION

In this study, three isolation techniques were used to isolate species of *Fusarium* from mangrove soil samples. Of the three techniques, the smallest number of isolates was obtained using soil dilution, perhaps because of the distribution of conidia in the diluted soil. The conidia may not be distributed as evenly in the dilutions as other microorganisms or propagules such as spores, hyphae and

Table 1: Texture, pH, moisture and *Fusarium* species isolated from mangrove soil samples using the soil dilution, direct isolation and debris isolation techniques.

Soil sample	Texture	pH	Moisture (%)	Isolation technique / *Fusarium* species		
				Soil dilution	Direct isolation	Debris isolation
M1	silty clay loam	7.32	5.0	*F. solani* (1)	*F. solani* (3)	*F. solani* (8)
M2	silty clay loam	7.49	5.0	–	–	*F. solani* (1)
M3	silty clay loam	7.52	6.0	–	*F. solani* (2)	*F. solani* (3), *F. oxysporum* (2)
M4	silty clay loam	7.39	5.0	–	–	–
M5	silty clay loam	7.23	6.0	*F. solani* (1)	*F. solani* (4)	*F. solani* (7), *F. verticillioides* (1)

Note: The numbers in parentheses denote the number of strains isolated

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

Characteristic	*Fusarium* species		
	F. solani	*F. oxysporum*	*F. verticillioides*
Microconidia	abundant in aerial mycelial, 0–2 septate, oval to kidney-shaped	abundant in aerial mycelial, 0–1 septate, obovoid to kidney-shaped, aerial mycelial with false heads	microconidia in long chains, 0–1 septate, oval, obovoid and pyriform
Macroconidia	abundant in sporodochia, 3–7 septate, straight to slightly sickle, stout, slightly hook or blunt rounded at the apical, distinctly notched or barely notched basal cell	in sporodochia, three septate, slightly sickle shaped with thin walls, blunt or slightly hook apical cell and foot-shaped or barely notched basal cell	not found
Conidiophore	long monophialides and branched monophialides	short monophialides	monophialides
Chlamydospore	present in pairs, cluster and singly	present singly and in pairs	not found
Pigmentation	light orange, yellowish, light brownish to dark brownish	pale violet to dark violet, white purplish	grayish orange and dark violet
Growth rate	1.9±0.3 cm to 3.1±0.3 cm	3.5±0.3 cm to 4.0±0.3 cm	3.5±0.3 cm to 4.4±0.3 cm

bacteria. Additionally, actinomycetes may be concentrated on the surface of the dilution due to their light weight (Garrett 1981).

The soil dilution technique is the best-known method for the estimation of microorganisms distributed in soils, although this method did not yield sufficient colonies for statistically valid enumeration of CFU per gram of soil (Koch 1994; Foght & Aislabie 2005). Only 4 to 12 colonies formed per plate from each of 4 soil samples (M1, M2, M3 and M5), whereas 30 to 300 would be required for a statistically valid result. Hence, the CFU method may not be precise enough to estimate the distribution of *Fusarium* species in the soil samples.

The direct isolation technique could be an effective and simple isolation method if the objective is to recover a large number of fungal isolates from different genera (Leslie and Summerell 2006). Nine isolates of *F. solani* were obtained via this method from three soil samples (M1, M3 and M5). This low number could be due to high contamination from non-*Fusarium* fungi or bacteria present in the soil samples. Moreover, it is possible the soils were not evenly distributed on the surface of the PCNB media and fungal colonies overlapped.

Most of the species of *Fusarium* obtained in this study were obtained via the debris isolation technique. The debris in soil samples were dried leaves, roots and branches; these substrates often serve as habitats for saprophytic and parasitic fungi. Roots are rich sources of nutrients and provide two favourable habitats, namely the root external surface, or rhizoplane, and the rhizosphere, a nutrient-enhanced zone around the root resulting from exudates of the plant cells (Curl & Truelove 1986; Bansal & Mukerji 1996). Branches and wood are also readily available carbon sources and are suitable substrates for fungal growth.

The mangrove soil samples were soggy and soft with a slightly alkaline to neutral pH and high moisture content, typical of mangrove soils. Similar pH and moisture content values were also obtained by Tariq *et al*. (2008), who measured pHs in the range of 7–10 and a moisture content of 8%–9%. The high moisture content is likely because the mangrove swamp is an active tidal area. The sea water provides moisture and temperature control with every cycle of the tides.

The distribution of *F. solani* is cosmopolitan, and it can be found in native soils worldwide (Joffe & Palti 1977; Marasas *et al*. 1988; Leslie *et al*. 1990; Jeschke *et al*. 1990; Burgess & Summerell 1992). Therefore, it is not surprising that *F. solani* was the most prevalent species recovered from the mangrove soil samples. In a related study, Tariq *et al*. (2006) isolated *F. solani* from different parts of mangrove plants, including the *Avicennia marina* rhizosphere (Tariq *et al*. 2008). In addition to mangrove soils, *F. solani* has been recovered from salt marshes, which are also anaerobic areas of high of salinity (Pugh 1962).

Like *F. solani*, *F. oxysporum* is also widely distributed in various types of soil, such as those of the Arctic (Kommendahl *et al*. 1988), the desert (Joffe & Palti 1977), cultivated and temperate soils (McMullen & Stack 1983; Latiffah *et al*. 2007). *F. oxysporum* was also one of the fungal species isolated from mangrove mud in Kagh Islands, India (Rai *et al*. 1966). Stover (1955) reported that *F. oxysporum* could survive in submerged anaerobic marsh soils, another extreme environment. It has been reported that *F. oxysporum* can be dispersed through wind, soils, seeds and infected planting material (Garibaldi *et al*. 2004),

which could explain the occurrence of *F. oxysporum* in the mangrove environment.

Only one isolate of *F. verticillioides* was recovered via the debris isolation technique. *F. verticillioides* is widely distributed worldwide and has been reported to occur in desert soils (Joffe & Palti 1977). The species could be dispersed through infected plant materials and wind dispersal, perhaps accounting for its occurrence in mangrove soils. *F. verticillioides* has a high survival rate and persists in host residues on the soil surface for up to 900 days under cool and dry conditions (Liddell & Burgess 1985).

In addition to the three species of *Fusarium* recovered in this study, other species of *Fusarium* have also been recovered from the mangrove environment. *G. fujikuroi* (teleomorph state of *F. fujikuroi)* was reported as one of the most prevalent species in mud samples from Red Sea mangroves in Egypt (Khallil *et al*. 1991). *Fusarium culmorum* and *F. proliferatum* were isolated from water and mud samples from an *A. marina* mangrove area (Mehdi & Saifullah 2000). Tariq *et al*. (2008) also recovered *F. semitectum* from the rhizosphere of *A. marina*.

Occurrences of the genus *Fusarium* in mangrove soils and plant debris suggest that the fungus can tolerate the extreme muddy, waterlogged, and aerobic environment of the mangrove swamp. Moreover, mangrove soils are rich in substrates, ranging from the leaves of mangrove trees, lignocellulose in the form of twigs and branches, fruits that fall into the water, roots and pneumatophores. All of these substrates sustain a wide range of fungi, including species of *Fusarium* (Jones & Alias 1997). The extensive colonisation of *Fusarium* on mangrove plants may explain the high recovery observed via the debris plating technique. The occurrence of *Fusarium* in soil samples and plant debris could also be due to its ability to produce chlamydospores and resistant hyphae that can survive in the soil under adverse environmental conditions (Burgess 1981).

In conclusion, by using soil dilution, direct isolation and debris isolation techniques, three species of *Fusarium* were recovered from mangrove soil samples and plant debris in Kampung Pantai Acheh, Balik Pulau, Pulau Pinang, Malaysia. Based on identification using morphological characteristics, the three species of *Fusarium* were identified as *F. solani* (91%), *F. oxysporum* (6%) and *F. verticillioides* (3%). This indicates that species of *Fusarium* can tolerate the extreme environments of the mangrove habitat.

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REFERENCES

Bansal M and Mukerji K G. (1996). Root exudaties in rhizophere biology. In K G Mukerji and V P Singh (eds.). *Concepts in applied microbiology and biotechnology.* New Delhi: Aditya Books, 97–100.

Burgess L W and Summerell B A. (1992). Mycogeography of *Fusarium*: Survey of *Fusarium* species from sub-tropical and semi-arid grassland soils from Queensland, Australia. *Mycological Research* 96: 780–784.

Burgess L W. (1981). General ecology of the Fusaria. In P E Nelson, T A Toussoun and R J Cook (eds.). Fusarium: *Diseases, biology, and taxonomy*. University Park, USA: The Pennsylvania State University, 225–235.

Curl E A and Truelove B. (1986). *The rhizophere.* Berlin-Heidelberg: Springer-Verlag.

Foght J and Aislabie J. (2005). Enumeration of soil microorganisms. In R Margesin and F Schinner (eds.). *Manual of soil analysis: Monitoring and assessing soil bioremediation*. *Soil Biology 5*. Berlin-Heidelberg: Springer-Verlag.

Garibaldi A, Gilardi G and Gullino M L. (2004). Seed transmission of *Fusarium oxysporum* f. sp*. lactucae. Phytoparasitica* 32: 61–65.

Garrett S D. (1981). *Soil fungi and soil fertility: An introduction to soil mycology* 2nd ed. Oxford: Pergamon Press.

Geiser D M, Delmar Jimenez-Gasco, Kang S, Makalowska I, Veeraraghavan N, Ward T J, Zhang N, Kuldau G A and O'Donnell K. (2004). Fusarium-ID V. 1.0: A DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology* 110(4): 473–479.

Head K H. (1980). *Manual of soil laboratory testing*. New York: John Wiley & Sons.

Jeschke N, Nelson P E and Marasas W F O. (1990). *Fusarium* species isolated from soil samples collected at different altitudes in Transkei, Southern Africa. *Mycologia* 82: 727–733.

Joffe A Z and Palti J. (1977). Species of *Fusarium* found in uncultivated desert-type soils in Israel. *Phytoparasitica* 5: 119–122.

Jones E B G and Alias S A. (1997). Biodiversity of mangrove fungi. In K D Hyde (ed.). *Biodiversity of tropical microfungi.* Hong Kong: Hong Kong University Press, 71–91.

Kathiresan K and Bingham B L. (2001). Biology of mangrove and mangrove ecosystems. *Advances in Marine Biology* 40: 81–251.

Khallil A-R M A, El-Hissy F T and Bagy M M K. (1991). Mycoflora of mangroves of Red Sea in Egypt. *Folia Microbiologica* 36(5): 456–464.

Koch A L. (1994). Growth measurement. In P Gerhardt, R G E Murray, W A Wood and N R Krieg (eds.). *Methods for general and molecular bacteriology.* Washington DC: American Society for Microbiology Press, 248–277.

Kommedahl T, Abbas H K, Barnes P M and Mirocha C J. (1988). Prevalance and toxigenicity of *Fusarium* species form soils of Norway near the Arctic Circle. *Mycologia* 80: 790–794.

Latiffah Z, Mohd Zariman M and Baharuddin S. (2007). Diversity of *Fusarium* species in cultivated soils in Penang. *Malaysian Journal of Microbiology* 3(1): 27–30.

Leslie J F and Summerell B A. (2006). *The* Fusarium *laboratory manual* 1st ed. Ames, Iowa: Blackwell Publishing.

Leslie J F, Pearson C A S, Nelson P E and Toussoun T A. (1990). *Fusarium* species from corn, sorghum and soybean fields in central and eastern United States. *Phytopathology* 80: 343–350.

Liddell C M and Burgess L W. (1985). Survival of *Fusarium moniliforme* at controlled temperature and relative humidity. *Transactions of the British Mycological Society* 84: 121–130.

Marasas W F O, Burgess L W, Anelich R Y, Lamprecht S C and van Schalkwyk D J. (1988). Survey of *Fusarium* species associated with plant debris in South African soils. *South African Journal of Botany* 54: 63–71.

McMullen M P and Stack R W. (1983). *Fusarium* species associated with grassland soils. *Canadian Journal of Botany* 61: 2530–2538.

Mehdi F S and Saifullah S M. (2000). Species diversity and seasonal occurrence of fungi on seedlings of *Avicennia marina* (Forsk.) Vierh. *Pakistan Journal of Biological Sciences* 3(2): 265–268.

Nelson P E, Toussoun T A and Marasas W F O (1983). Fusarium *species. An illustrated manual for identification.* University Park, USA: The Pennsylvania State University Press.

Pugh G J F. (1962). Studies on fungi in coastal soils II. Fungal ecology in a developing salt marsh. *Transactions of the British Mycological Society* 45: 560–566.

Rai J N, Tewari J P and Mukerji K G. (1966). Mycoflora of mangrove mud. *Mycopathologia et Mycologia Applicata* 38: 17–31.

Stover R H. (1955). Flood fallowing for eradication of *Fusarium oxysporum f. cubense:* III. Effect of oxygen on fungus survival. *Soil Science* 80: 397–412.

Tariq M, Dawar S and Mehdi F S. (2008). Studies on the rhizosphere mycoflora of mangroves. *Turkish Journal of Botany* 32: 97–101.

_____. (2006). Isolation of fungi from *Avicennia marina*. *Pakistan Journal of Botany* 38(3): 805–810.

Thein S J. (1979). A flow diagram for teaching texture analysis by feel. *Journal of Agronomic Education* 8: 54–55.