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SHORT COMMUNICATION

Endophytic Fungi from Paddy

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Abstrak: Kulat endofit telah dipencilkan daripada bahagian berbeza tanaman padi (*Oryza sativa*) yang sihat. Genus yang paling lazim diperolehi adalah *Fusarium* diikuti oleh *Aspergillus, Curvularia*, *Penicillium*, *Gilmaniella* dan *Arthrobotrys foliicola. Fusarium* dan *Curvularia* didapati lebih banyak di dalam biji benih. *Aspergillus* dipencilkan kebanyakannya daripada bilah daun dan *Penicillium* daripada seludang daun. *Gilmaniella* dan *A. foliicola* pula hanya dipencilkan daripada akar dan bilah daun masing-masing. Sekumpulan kulat endofit yang diperolehi daripada tisu-tisu padi yang sihat menunjukkan kemungkinan sebahagian kulat tersebut merupakan patogen pendam dan sebahagian lagi boleh menjadi saprofit.

Kata kunci: Endofit, Kulat, Padi

Abstract: Endophytic fungi were isolated from different parts of healthy paddy plants (*Oryza sativa*). The most common endophytic fungal genus recovered was *Fusarium*, followed by *Aspergillus, Curvularia*, *Penicillium*, *Gilmaniella* and *Arthrobotrys foliicola. Fusarium* and *Curvularia* had higher occurrences in the seeds compared with the other fungi. *Aspergillus* was recovered mostly from leaf blades and *Penicillium* from the leaf sheath. *Gilmaniella* and *A. foliicola* were isolated only from the roots and leaf blade, respectively. The assemblage of endophytic fungi in healthy tissues of paddy plants may indicate that some of the fungi are possible latent pathogens and some may become saprophytic.

Keywords: Endophyte, Fungi, Paddy

Endophytic fungi colonise healthy plant tissues but do not always cause noticeable symptoms. This interaction is regarded as mutualistic (Carroll 1988). According to Schulz and Boyle (2005), however, this mutualistic interaction may only be temporary and is subject to change over time. Therefore, endophytic fungi could account for those fungi with an epiphytic phase as well as latent pathogens that live asymptomatically in the host plant for some time in their life (Petrini 1991).

In a mutualistic interaction, an endophyte in plant tissues obtains nutrients and protection from the plant host, while returning metabolites that contribute to host resistance against herbivores, pathogens and various abiotic stresses, thereby enhancing the plant's fitness in a harsh environment (Saikkonen *et al*. 1998; Redman *et al*. 2002). The stability of a mutualistic interaction may change, depending on several factors such as environmental

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stress, senescence of the plant and the plant defence response to infection (Schulz & Boyle 2005).

There have been numerous studies on endophytic fungi in agricultural crops, such as maize (Fisher *et al.* 1992), banana (Cao *et al*. 2002), coffee (Santamaria & Bayman 2005) and wheat (Larran *et al.* 2007), indicating that endophytic fungi are diverse in various crops. Paddy is one of the most important commercial crops planted in Malaysia, particularly for domestic consumption. There are relatively few studies focused on endophytic fungi colonising healthy paddy tissues. Thus, the objective of the present study was to assess the endophytic fungal assemblages in different parts of apparently healthy paddy plants.

Paddy samples were collected from three locations in Pulau Pinang: Sungai Burung, Balik Pulau; Permatang Pauh, Seberang Perai; Batu Dua, and Sungai Setar, Nibong Tebal. The plants collected were at reproductive stage, and a total of 10 plants were collected at each location. Only healthy plants displaying no disease symptoms or pest infestation were selected. The samples were processed within 48 h after sampling to avoid desiccation of the plant tissues.

For isolation of endophytic fungi, 20 plants, comprising plants from each of the selected locations, were selected. All parts of the paddy plants were thoroughly washed in running tap water for 24 h to remove debris and any soil particles adhered to the surface of the plants. Leaf sheath, leaf blade, stems and roots were cut into small pieces of about 5 mm. For leaf sheath, leaf blade and stems, 40 tissue pieces from each plant part were used for isolation. From the roots, 50 small pieces of tissue were used to isolate endophytic fungi.

The pieces of tissue were surface sterilised by immersion in 75% alcohol for 5 min, then 80% natrium hyphochlorite for 1 min; tissues were then washed in sterile distilled water for 1 min. Plant tissues were plated on the surface of potato dextrose agar (PDA) and peptone chloronitro benzene (PCNB) media. PCNB was used for the isolation of *Fusarium* species.

The plates were incubated at 27 ± 1°C for 1–10 days, or until mycelia growth from the tissues was observed. Mycelia growing out from the plant tissues were then sub-cultured on PDA to obtain pure culture and used for identification. The endophytic fungi were identified by examining the microscopic and macroscopic structures. Taxonomic fungal manuals of Ellis (1971), Barnett and Hunter (2006) and Leslie and Summerell (2006) were used for identification of the endophytic fungi.

Mycelia sterilia fungi were identified using internal transcribed spacer region of ribosomal DNA (ITS1-5.8S-ITS2). Polymerase chain reaction (PCR) and sequencing procedures, as well as the primer sequences used, were as previously described by White *et al*. (1990).

A total of 110 fungal isolates were recovered from different parts of the paddy plant. The occurrence of endophytic fungi in different parts of the plant was not tissue specific. From seeds, 40 fungal isolates were recovered. The number of isolates recovered from leaf blade, leaf sheath and stem were 32, 21 and 10, respectively. Only seven isolates were recovered from the roots (Table 1).

Table 1: Occurrence of endophytic fungi in healthy tissues of paddy.

Fungi	Plant tissues					
	Seed	Leaf blade	Leaf sheath	Stem	Root	Total number of isolates
Fusarium	25	5	5	3	5	43
Aspergillus	2	14	4	6	0	26
Curvularia	13	5	3	0	0	21
Penicillium	0	6	9	1	1	17
Gilmaniella	0	0	0	0	1	1
Arthrobotrys foliicola	0	2	0	0	0	2
	40	32	21	11	8	110

Five genera of fungi were identified based on macroscopic and microscopic structures, and one genus of mycelia sterilia was identified using sequences of the ITS1-5.8S-ITS2 regions. The most common fungal genus recovered was *Fusarium*, followed by *Aspergillus, Curvularia* and *Penicillium*. *Gilmaniella* was only isolated from the stem. Based on the ITS1-5.8S-ITS2 regions, the two mycelia sterilia isolates recovered from leaf blade were identified as *Arthrobotrys foliicola* (Fig. 1). The sequences of ITS1-5.8S-ITS2 regions of the 2 isolates showed 100% homology with *A. foliicola* (AFU51954).

Fusarium and *Curvularia* appeared to have higher occurrences in the seed compared with other fungi. On the other hand, *Aspergillus* was recovered in higher frequency in the leaf blade and *Penicillium* in the leaf sheath compared with other fungi. The uncommon endophytic fungi of *Gilmaniella* and *A. foliicola* were recovered from the root and leaf blade, respectively.

The endophytic fungi isolated from different parts of paddy could be saprophytic such as *Gilmaniella* and *A. foliicola* or potentially pathogenic from the genus *Fusarium*, *Aspergillus, Curvularia* and *Penicillium*. However, these fungal genera are also well-known endophytes in healthy tissues of several plants (Fisher & Petrini 1992; Tian *et al.* 2004; Geris dos Santos *et al*. 2003).

Plant pathogenic species of *Fusarium* have been known to be associated with several types of paddy disease such as bakanae, seedling rot and root rot. The occurrence of *Fusarium* in paddy plants implies that this fungus is a latent pathogen. Leslie *et al*. (1990) reported asymptomatic infections by species of *Fusarium* in maize, sorghum and soybean. Two species of *Fusarium*, *F. equiseti* and *F. oxysporum* have been isolated from healthy paddy plants by Fisher and Petrini (1992).

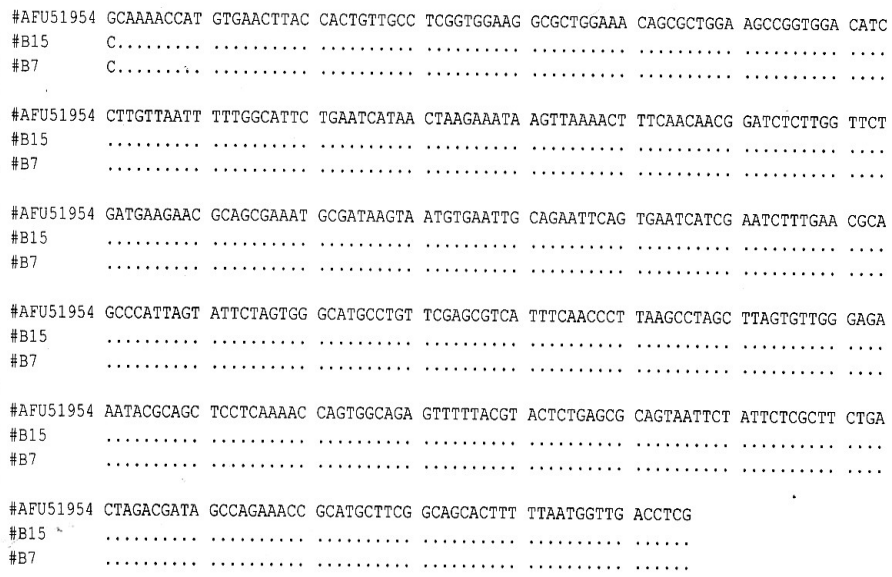

Figure 1: Sequence alignment of two mycelia sterilia isolates (B15 and B7) with accession AFU51954 (*A. foliicola*).

In the present study, *Fusarium* was frequently isolated from the seeds. Species of *Fusarium* have been reported to be one of the most common fungi recovered from paddy grains (Abdel-Hafez *et al.* 1987), paddy and milled rice (Tonon *et al*. 1997) and paddy seeds (Pacin *et al.* 2002). In addition to paddy seed, species of *Fusarium* have been isolated from soybean seeds (Pacin *et al*. 2002) and cowpea seeds (Rodrigues & Menezes 2005).

Besides *Fusarium*, *Aspergillus* and *Penicillium* were also among the most common endophytic fungal genera recovered from healthy paddy plant in China (Tian *et al*. 2004). From healthy leaves and roots of paddy plants, *Penicillium chrysogenum* and *F. oxysporum* were frequently isolated (Shankar Naik *et al*. 2009). Rodolfi *et al*. (2006) reported that endophytic colonisation of seeds by *Penicillium, Fusarium* and *Aspergillus* were detected on more than one Italian rice cultivar. In addition to paddy, endophytic species of *Aspergillus* and *Penicillium* were also recovered from banana leaves and roots (Cao *et al.* 2002) and leaves of *Plumeria rubra*, a tropical deciduous tree (Suryanarayanan & Thennerasan 2004).

Isolates of *Curvularia* were mostly recovered from paddy seeds. This genus is a well-known pathogen of paddy seed (Estrada & Sandoval 2004) and is among the mycoflora most frequently isolated from paddy seeds (Tonon *et al*. 1997; Abdel-Hafez *et al.* 1987).

Two isolates of mycelia sterilia isolated from the leaf blade were identified as *A. foliicola*. Species of *Arthrobotrys* are nematophagous fungi that can exhibit endophytic and free living life styles in rhizophere soils (Lopez-Llorca *et al.* 2006). Two species of *Arthrobotrys* have been reported to be endophytic in plants. *A*. *conoides* has been recovered from the bark of *Crataeva magna* (three leave caper) (Nalini *et al*. 2005), and *A. oligospora* was shown to endophytically colonise barley roots (Bordallo *et al.* 2002).

Gilmaniella was isolated from paddy stem. Endophytic *Gilmaniella* has been isolated from healthy tissues of root, stem, leaves and fruits of *Melia azedarach* (Geris dos Santos *et al.* 2003).

Interactions between an endophyte and host may change over time (Saikkonen *et al.* 1998; Schulz & Boyle 2005). The endophyte may undergo physiological alterations, from a mutualistic to a pathogenic interaction, or vice versa, depending on several factors such as drought, excessive humidity, stress and poor nutrient supply (Millar 1980; Fisher & Petrini 1992). For most endophytic fungi, the types of interactions between the microbe and the host are described as an interaction at a particular point of time or momentary status (Schulz & Boyle 2005). However, an endophytic fungus could act as a latent pathogen in the plant tissues until changes in environmental factors or decline in host defence mechanisms allow the endophyte to become pathogenic (Bayman 2007). Thus, the assemblage of endophytic fungi from paddy plants may indicate that some of the fungi such as *Fusarium*, *Aspergillus, Curvularia* and *Penicillium* are possible latent pathogens, a suggestion put forth by Fisher and Petrini (1992). The other two fungi, *Gilmaniella* and *A. foliicola,* could become saprophytic at a later stage of plant growth, but their roles in the paddy plant are not clear and need further study.

In conclusion, six endophytic fungal genera, including *Fusarium*, *Aspergillus, Curvularia*, *Penicillium*, *Gilmaniella* and *A. foliicola,* were recovered from different parts of paddy plants, indicating that an assemblage of endophytic fungi occurs in the tissues of the paddy plant.

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