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Diversity of Endophytic Fungi Isolated From Plant Leaves of Deciduous Dipterocarp Forest in Tak Province

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ABSTRACT

Endophytic fungi were isolated from the leaves of nine tree species from deciduous dipterocarp forest in Tak province during the wet season of 2007 and the dry season of 2008. The tree species were: *Shorea obtusa*, *Shorea siamensis*, *Careya sphaerica*, *Morinda elliptica, Anthocephalus chinensis, Grewia eriocarpa, Terminalia chebula, Fermandoa adenophylla* and *Erythrina subumbrans* Healthy plant leaves were prepared by a surface sterilization technique and placed on potato dextrose agar (PDA). Fungal identification was based on colony and morphological characteristics. The frequency of the fungal endophytes isolated was calculated in order to compare the distribution of fungal diversity. Two hundred and thirty one endophytic fungi were isolated from 340 leave segment samples. The diversity of endophytic fungi in the wet season was greater than in the dry season. Mycelia sterilia sp.2 was the most frequent isolate from the leaves of the different plant host species. Moreover, typical endophytic fungi genera, such as *Phomopsis* sp., *Nigrospora* sp., *Fusarium* sp., *Pestalothiopsis* sp. and *Xylaria* sp. were commonly found in this deciduous dipterocarp forest.

Key words: endophytic fungi, diversity of endphytic fungi, deciduous dipterocarp forest

INTRODUCTION

Recently, the need for new, useful compounds for curing human diseases has become ever increasing because of the development and spread of drug-resistant pathogens. Moreover, anticancer drugs are also required due to the high worldwide mortality rate. With the discovery of the anticancer drug, paclitexel (Taxol), from *Pestalotiopsis microspora*, a fungus that colonizes the Himalayan yew tree *Taxus wallichiana* (Strobel *et al*., 1996), endophytic fungi from plants have become a new traditional source of bioactive compounds.

Endophytes are all organisms that live inside plant tissue for at least part of their life cycle without causing any disease symptoms in the host (Petrini, 1991). The symptomless nature of endophyte occupation in plant tissue has prompted a focus on symbiotic or mutualistic relationships between endophytes and their hosts. The biodiversity observed in endophytes suggests that they can also be aggressive saprophytes or opportunistic pathogens (Bacon and White, 2000).

Hawksworth and Rossman (1987) estimated that there may be as many as one million different fungal species, yet only about 100,000 have been described. As more evidence

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accumulates, estimates of the actual number of fungal species keep rising. For example, Dreyfuss and Chapela (1994) estimated that there may be at least one million species of endophytic fungi alone. It seems obvious that endophytes are a rich and reliable source of genetic diversity and novel, undescribed species.

Currently, the study of bioactive compounds from endophytic fungi is carried out in association with various bioactivities, such as immunosuppressive agents, anticancer agents and antimicrobial agents. Moreover, study of the biodiversity of endophytic fungi is important because it suggests chemical diversity. There is constant chemical innovation existing in ecosystems as part of the evolutionary race to survive (Redell and Gordon, 2002). It is accepted that endophytes are ubiquitous and are an important component of fungal diversity, even though they are host-restricted to the tropics. Deciduous dipterocarp forest is one such interesting tropical site. It shows great diversity and commonly occurs in Southeast Asian countries, including Thailand. This type of forest has an open canopy and is composed of small- to medium-size xeric species. Most of the tree species have broad leaves that are able to capture fungal spores.

The main objective of this study was to examine the diversity of endophytic fungi in a deciduous dipterocarp forest in Tak province during the wet and dry season.

MATERIALS AND METHODS

Plant samples collection

During the wet season of 2007 and the dry season of 2008, mature, healthy plant leaves were collected by sampling from different parts of trees (Table 1) growing in a deciduous dipterocarp forest in Tak province, at location north 16° 44' 35.07" and east 98° 48' 51.945". All plant material was placed in clean, sterile polythene bags and immediately transferred to the laboratory in an icebox.

Samples were stored at 5°C and isolated for endophytic fungi within 48 h after collection.

Isolation and identification of endophytic fungi

Leaves were carefully washed with running tap water and then were cut into small pieces of approximately 5×5 mm^2. The specimens were surface sterilized using the method described by Blodett *et al*. (2000); the samples were immersed in 95% ethanol for 1 min, 10% sodium hypochlorite solution for 5 min, 95% ethanol for

Table 1 List of plant species collected for isolation of endophytic fungi.

No.	Plant species	Collection segment code		Average leaf surface (cm^2)
		Dry	Wet	
1	*Shorea obtusa*	SOD	SOW	70
2	*Shorea siamensis*	SSD	SSW	67
3	*Careya sphaerica*	CSD	CSW	300
4	*Morinda elliptica*	MPD	MPW	70
5	*Anthocephalus chinensis*	ACD	ACW	220
6	*Grewia eriocarpa*	GED	GEW	80
7	*Fermandoa adenophylla*	FAD	FAW	46
8	*Terminalia chebula*	TCD	TCW	82
9	*Erythrina subumbrans*	ESD	ESW	48

The first two letters in the collection segment code denote the host code of the plant species, while the third letter denotes the season during which the fungus was isolated (W = wet, D = dry).

30 seconds and then rinsed in sterile water. The sterile specimens were placed on potato dextrose agar (PDA) in a Petri dish and incubated at room temperature. Fungal colony development was visually inspected daily. Fungal mycelia were subcultured onto new media until pure cultures were obtained.

The fungal isolates were examined under a light microscope and identification to the genus level was undertaken by referring to the key described by Barnett and Hunter (1987) and Von Arx (1978).

The colonization frequency (CF%) of a single endophytic species was calculated using Equation 1, (Gond *et al*., 2007):

$$CF\% = (N_{col} / N_t) \times 100 \quad (1)$$

where, N_{col} = number of segments colonized by each fungus

N_t = total number of segments studied

The similarity of endophytic fungi between seasons was calculated and compared according to the idex described by Jaccard (1901) (Equation 2):

$$\% \text{ similarity} = C / (A+B+C) \times 100 \quad (2)$$

where, A = total number of species in season

B = total number of species in season

C = total number of species in both seasons

RESULTS

A total of 95 isolates belonging to 7 genera and 20 species, and 136 isolates representing 13 genera and 21 species occurred as endophytes in the deciduous dipterocarp forest in Tak province during the dry and wet season, respectively. It was not possible to collect the leaves of *Erythrina subumbrans* in the dry season because leaf shed had already occurred. The endophytic fungal colonization frequency in the wet and dry season was 75.5 and 59.3% respectively (Figure 1).

Mycelia sterilia sp.2 was the most commonly found, being present on all plant hosts. Moreover, typical endophytic fungi genera observed were: *Phomopsis* sp., *Nigrospora* sp., *Fusarium* sp., *Pestalothiopsis* sp., *Xylaria* sp., *Glomellera* sp. and *Phyllosticta* sp., although 38% percent of those were mycelia sterilia (Table 2). The diversity of endophytic fungi between the dry and wet seasons differed. *Paecilomyces* sp.2, *Phomopsis* sp. 8, mycelia sterliria sp.1 and mycelia sterliria sp.2 were the only isolates found in both seasons.

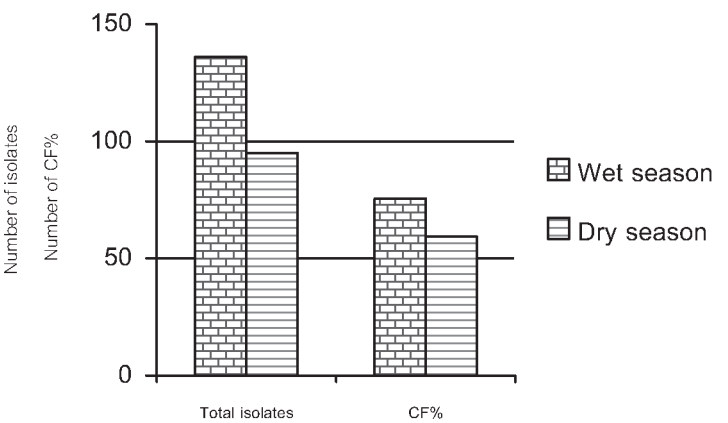

Figure 1 Comparison of the total isolates and colonization frequency of endophytic fungi from deciduous dipterocarp forest in Tak province during the dry and wet season.

Table 2 Colonization frequency (CF%) of endophytes from nine different host-tree species in deciduous dipterocarp forest in Tak province.

Endophyte	SO		SS		CS		ME		AC		TC		GE		FA		ES		Total
	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	CF%
Collectotrichum sp.	-	-	-	-	45	-	10	-	-	-	-	-	-	-	-	-	-	-	55
Glomerella sp.	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	5
mycelia sterilia sp.1	-	-	-	-	10	5	30	-	-	-	-	-	-	-	-	-	25	-	70
mycelia sterilia sp.2	45	-	15	-	-	-	20	-	20	15	25	-	5	-	-	-	35	-	180
mycelia sterilia sp.3	-	-	-	-	-	5	-	5	-	-	-	-	-	-	-	-	-	-	10
mycelia sterilia sp.4	-	-	-	-	-	-	-	-	25	-	-	-	-	-	10	-	-	-	35
mycelia sterilia sp.5	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	5
mycelia sterilia sp.6	-	-	-	-	-	-	-	-	-	-	15	-	-	-	10	-	-	-	25
mycelia sterilia sp.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	10
Nigrospora sp.	-	-	-	-	40	-	-	-	15	-	-	-	5	-	15	-	20	-	95
Paecilomyces sp.1	-	25	-	25	-	30	-	-	-	35	-	40	-	-	-	15	-	-	170
Paecilomyces sp.2	35	-	-	-	-	15	-	25	-	-	-	-	-	35	-	15	-	-	125
Paecilomyces sp.3	-	5	-	30	-	10	-	5	-	-	-	5	-	-	-	-	-	-	55
Pestalotiopsis sp.	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	10
Phomopsis sp.1	-	-	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15
Phomopsis sp.2	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
Phomopsis sp.3	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	5
Phomopsis sp.4	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	5
Phomopsis sp.5	-	-	-	-	-	-	-	-	-	-	-	-	10	-	15	-	-	-	25
Phomopsis sp.6	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	10
Phomopsis sp.7	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	-	-	10
Phomopsis sp.8	-	5	-	-	-	-	-	-	-	-	-	5	5	20	-	-	-	-	35
Phyllosticta sp.	-	-	15	-	-	-	-	-	10	-	25	-	-	-	-	-	-	-	50
Xylaria sp.1	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Xylaria sp.2	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
Xylaria sp.3	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10

Table 2 (continued).

Endophyte	SO		SS		CS		ME		AC		TC		GE		FA		ES		Total
	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	CF%
Xylaria sp.4	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Xylaria sp.5	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Xylaria sp.6	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Xylaria sp.7	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
Xylaria sp.8	-	-	-	-	-	15	-	-	-	-	-	-	-	-	-	-	-	-	15
Xylaria sp.9	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	5
Xylaria sp.10	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	10
Xylaria sp.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	10
Xylaria sp.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	5
Xylaria sp.13	-	-	-	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-	20
Xylaria sp.14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-	15
Total CFU%	80	70	65	70	115	95	60	35	75	50	75	55	55	55	65	45	90	-	1155
Total number of endophytes	16	14	13	14	23	19	12	7	15	10	15	11	11	11	13	9	18	-	231

Refer to Table 2 for host codes SO = *Shorea obtusa*, SS = *Shorea siamensis,* CS = *Careya sphaerica,* ME = *Morinda elliptica*, AC = *Anthocephalus chinensis,* GC = *Grewia eriocarpa*, FA = *Fermandoa adenophylla,* TC = *Terminalia chebula,* EV = *Erythrina subumbrans* (*D* = dry season ; *W* = wet season). CFU = Colony forming unit.

DISCUSSION

The colonization frequency was used to compare diversity. There was a higher frequency of endophytic fungi in the wet season than in the dry season. Moreover, the distribution of the endophytic fungi in the wet season was also greater than in the dry season (Table 2). This result suggested that moisture content was one of the main factors that influenced diversity. The rainfall in the wet season averaged 8.8 mm/day and there was no rainfall in the dry season. Wet conditions are favorable to fungal sporulation and increasing infective fungi (Wilson, 2000).

Two hundred and thirty-one fungal isolates were obtained and were identified to the genus level and 40 morphotaxa were classified. The most frequently isolated genus was *Paecilomyces* sp.1 in the dry season and mycelia sterilia sp.2 in the wet season. The highest fungal colonization frequency and diversity of taxa was obtained with *Careya sphaerica*, which has the largest leaf area and offers much greater surface area for inoculum capture (Mekkamol, 1998).

Moreover, coelomycetes, such as *Phomopsis* sp., *Xylaria* sp. and *Phyllosticta* sp., were also found. The diversity of endophytic fungi isolated in Tak province was lower than that of endophytic fungi isolated in the Doi Suthep-Pui area (Lumyong *et al*., 1998). The similarity index showed that the endophytic fungi in both seasons were 10.5% similar. Therefore, endophytic fungal communities in the deciduous dipterocarp forest in Tak province were distinctly associated with the host plants. Factors that might cause this phenomenon were assumed to include variations in vegetation type and environmental parameters, such as temperature, rainfall and humidity.

Xylariaceous fungi and mycelia sterilia were unable to be identified in this forest type, probably due to the lack of spore formation in the media. Fourteen groups of xylariaceous fungi and eight groups of mycelia sterilia were not differentiated on potato dextrose agar (PDA), Sabouraud dextrose agar (SDA) or malt extract agar (MEA). Therefore, a molecular technique will need to be used to obtain further identification of the groups of mycelia sterilia and xylariaceous fungi, which is expected to be more effective than the procedures used in the present study.

CONCLUSION

In this study, the leaves from each host were collected during the wet season of 2007 and the dry season of 2008 to investigate any seasonal effects. The number of isolates recovered depended on environmental factors, such as rainfall and humidity.

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