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Full Length Research Paper

Endophytic *Pestalotiopsis* species associated with plants of Palmae, Rhizophoraceae, Planchonellae and Podocarpaceae in Hainan, China

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A survey of the endophytic *Pestalotiopsis* associated with 27 plant species belonging to four families in Hainan Province was carried out from 2007 to 2008. Colonization frequencies of endophytic *Pestalotiopsis* species varied in the host plant's tissues, sites and natural environmental conditions. Species composition of endophytic *Pestalotiopsis* varied in different families of plants. A total of 43 endophytic *Pestalotiopsis* species were isolated, of which 23, 11, 9 and 8 species were obtained from families of Palmae, Rhizophoraceae, Podocarpaceae and Planchonellae, respectively. The species of *Pestalotiopsis* isolated from different hosts in Palmae family varied from 1 to 7. The colonization frequencies of endophytic *Pestalotiopsis* varied from environmental factors. The colonization frequencies of endophytic *Pestalotiopsis* in dry years were lower than that in usual years. Shannon-Wiener index of endophytic *Pestalotiopsis* in Palmae, Rhizophoraceae, Podocarpaceae and Planchonellae changed from 1.4775 to 2.5013. Evenness index changed from 0.3624 to 1.0431. Richness index of endophytic *Pestalotiopsis* had no correlation with Shannon-Wiener index and evenness index. The coefficient of community of endophytic *Pestalotiopsis* among the four plant families was less than 0.5, showing low similarity.

Key words: Endophytic *Pestalotiopsis*, colonization frequencies, diversity, host preference, richness index.

INTRODUCTION

Fungal endophytes have been characterized by their ability to produce apparently harmless infections in living plant tissues (Carroll and Carroll, 1978; White and Cole, 1985; Tejesvi et al., 2009). Extensive surveys in a wide variety of plants indicated that endophytes are apparently ubiquitous. But the researches of endophytic fungi were mostly concentrated in the northern hemisphere and temperate zones. Recent study of endophyte in tropical region begun with the investigation of endophyte in *Manilkara bidentata* and mangrove wild legume *canavalia cathartica*, which indicated that endophytic fungi were important community among fungi (Lodge et al., 1996; Anita and Sridhar, 2009). Although, investigations of endophytic fungi in some tropical plants were carried out, only several or even one plant species were involved in each study (Ananda and Sridhar, 2002; Kelemu et al., 2003; Maria and Sridhar, 2003; Gao et al., 2005; Suryanayanan et al., 2005; Rodriguez et al., 2009). Extensive surveys with the plants in the tropical area in China have not been done.

Endophytic *Pestalotiopsis* has been considered as a main part of the endophytic fungi community in nature (Strobel et al., 1996, 1997; Okane et al., 1997, 1998;

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Cannon and Simmons, 2002; Toofanee and Dulymamode, 2002; Kumar and Hyde, 2004; Photita et al., 2004; Wei and Xu, 2004; Rodriguez et al., 2009). Diversity analysis of endophytic *Pestalotiopsis* would join the research community composition and species distribution of endophytic fungi.

The regions of tropical forest were considered as the most abundant area of fungal resource (Bills and Polishhook, 1994; Gilbert and Sousa, 2002). Hainan Island lies in South China Sea, latitude 18°16' and 20°23', where the abundant plant, water, and heat resources in tropic rain forest provide basic condition for the development and multiplication of endophytic *Pestalotiopsis*. The aim of this paper was to demonstrate the species diversity of endophytic *Pestalotiopsis* on plants of Palmae, Rhizophoraceae, Podocarpaceae and Planchonellae in the tropical region, of Hainan Province, China.

MATERIALS AND METHODS

Sample collection

The healthy leaves and twigs were collected from the Xinglong Tropical Botanical Garden, the Danzhou Tropical Botanical Garden, the Jianfengling Natural Forest Reserve and the Dongzaigang Mangrove Reserve in Hainan Province in April 2007 and 2008.

Isolation and identification

The leaves and twigs were separated from their branches and washed with running tap water, surface sterilized with 75% ethanol (60 s), 1.3% NaClO (5 min) and 75% ethanol (30 s) (Wei and Xu, 2004). Samples were washed three times with sterilized water, cut into pieces of 1 cm long and placed on PDA medium. The plant tissues were incubated at 25°C for 3-20 days and were checked regularly. Pure fungal cultures were obtained by single spore isolation following the methods outlined by Lacap et al. (2003) and Liu et al. (2007).

Hyphal tip from the colony margin was transferred into new Petri-dish with PDA medium. When colony grew up to 2 cm in diameter, the autoclaved segment of the carnation leaf (*Dianthi caryophylii* L.) was added to the culture to promote sporulation (Fisher et al., 1982; Strobel et al., 1996). *Pestalotiopsis* species were identified according to the morphological descriptions of Steyaert (1949), Sutton (1980) and Nag Raj (1993).

The living cultures of *Pestalotiopsis* species were deposited in the Institute of Biotechnology, Zhejiang University, Hangzhou and the China General Microbiological Culture Collection Center in Beijing, China.

Data analysis

Colonization frequency was calculated as the number of plant tissue segments colonized by *Pestalotiopsis* species divided by the total number of segments assessed (Liu et al., 2007). The Shannon-Wiener diversity index (*H*) was used to estimate the species diversity of the fungal assemblages recovered from a particular type of sample (leaf and twig) and from different sampling sites. The *H´* was calculated according to the following formula:

$$H' = -\sum_{i=1}^{k} pi \times \ln pi$$

Where, *k* is the total number of fungal species, and *pi* is the proportion of individuals that species *i* contributes to the total (Pielou, 1975).

To evaluate the degree of community similarity of endophytic *Pestalotiopsis* species between two sampling sites, Sorenson's coefficient (C_S) was employed and calculated according to the following formula:

$C_S = 2j/(a+b)$,

Where, *j* is the number of endophytic *Pestalotiopsis* species that coexisted in both sampling sites, *a* is the total number of endophytic *Pestalotiopsis* species in one sampling site, and *b* is the total number of endophytic *Pestalotiopsis* species in the other sampling site (Liu et al., 2007; Tejesvi et al., 2008).

Statistical analysis was made by DPS (Data Processing System) version 7.05 professional edition.

RESULTS

Colonization frequencies of endophytic *Pestalotiopsis* in different host plants

The colonization frequencies of endophytic *Pestalotiopsis* species in seven plants of Palmae, nine mangrove plants, five plants of Planchonellae and six plants of Podocarpaceae are shown in Figure 1.

The colonization frequency of endophytic *Pestalotiopsis* species in the leaves of Palmae plants varied from 0.8 to 27.2% (Figure 1), and the colonization of endophytic *Pestalotiopsis* species in leaves of mangrove plants varied from 3.3 to 40.0% (Figure 1). Five plants of Planchonellae were selected to analyze the colonization frequency of endophytic *Pestalotiopsis* (Figure 1). The colonization frequency of the endophytic *Pestalotiopsis* in twigs of *Manilkara zapota* was significantly higher (23.3%) than those of other plants, but there was no significant difference among the other plants. The colonization frequency of Podocarpaceae plants were 1.7, 15, 16.7, 8.3, 20 and 10% in *Podocarpus fleuryi*, *Podocarpus imbricatus*, *Dacrydium pierrei*, *Podocarpus macrophyllus*, and *Nageia nagi*, respectively (Figure 1).

Colonization frequencies of endophytic *Pestalotiopsis* in different years

The colonization frequencies of endophytic *Pestalotiopsis* species in seven common plants of Palmae were analyzed between 2007 and 2008 (Figure 2). The colonization frequencies of endophytic *Pestalotiopsis* species in *Neodypsis decaryi* were 50%. However, the colonization frequencies in 2008 were only 4.4%. The other plants of Palmae behaved in the same trend in varied degree except in *Hyophorbe lagenicaulis* and *Caryota ochlandra*. No endophytic *Pestalotiopsis* species was isolated from *C. ochlandra*, *Zalacca wallichiana* and

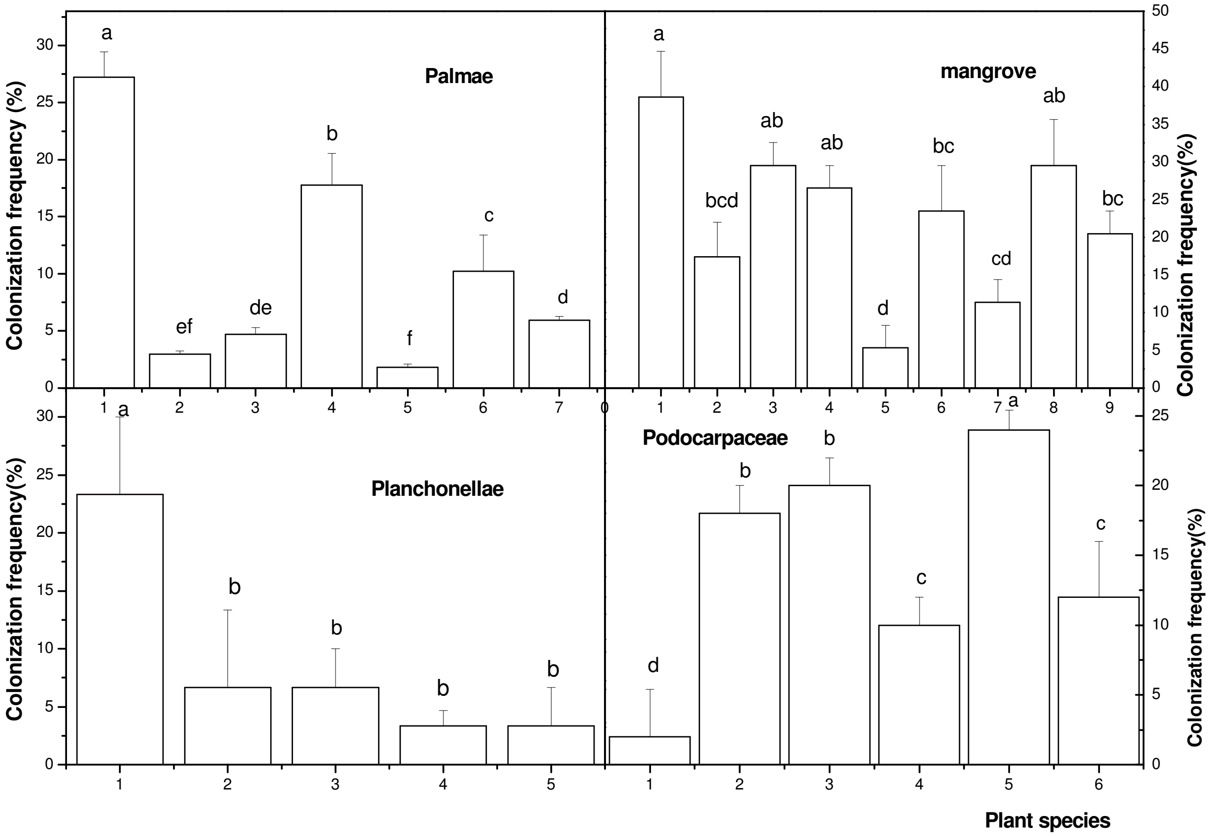

Figure 1. Colonization frequencies of endophytic *Pestalotiopsis* in leaves of different host plants; Palmae: 1. *Neodypsis decaryi*; 2. *Hyophorbe lagenicaulis*; 3. *Wodyetia bifurcate*; 4. *Chamaedorea oblongata*; 5. *Caryota ochlandra*; 6. *Zalacca wallichiana*; 7. *Chrysalidocarpus lutesens*. Mangrove: 1. *Bruguiera gymnorrhiza*; 2. *Kandelia candel*; 3. *Bruguiera sexangula*; 4. *Bruguiera sexangula*; 5. *Ceriops tagal*; 6. *Rhizophora mucronata*; 7. *Hibiscus tiliaceus*; 8. *Aegiceras coniculatum*; 9. *Xylocarpus granatum*. Planchonellae: 1. *Manilkara zapota*; 2. *Madhuca hainanensis*; 3. *Mimusops elengi*; 4. *Synsepalum dulcificum;* 5. *Lucuma nervosa*. Podocarpaceae: 1. *Podocarpus fleuryi*; 2. *Podocarpus imbricatus*; 3. *Dacrydium pierrei*; 4. *Podocarpus macrophyllus*; 5. *Nageia nagi*; 6. *Podocarpus macrophyllus*.

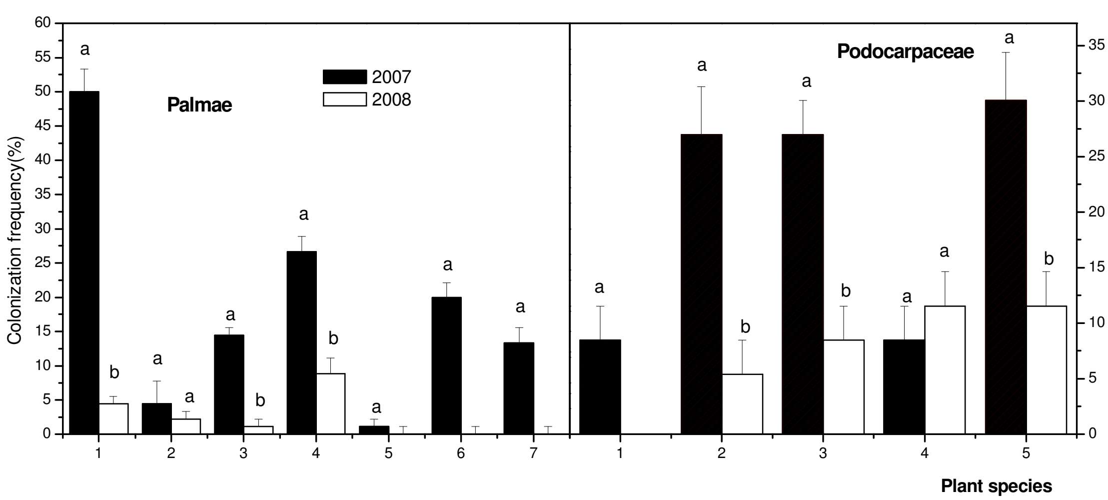

Figure 2. Colonization frequencies of endophytic *Pestalotiopsis* of the leaves in different years. Palmae: 1. *Neodypsis decaryi*; 2. *Hyophorbe lagenicaulis*; 3. *Wodyetia bifurcate*; 4. *Chamaedorea oblongata*; 5. *Caryota ochlandra*; 6. *Zalacca wallichiana*; 7. *Chrysalidocarpus lutesens*. Podocarpaceae: 1. *Podocarpus fleuryi*; 2. *Podocarpus imbricatus*; 3. *Dacrydium pierrei*; 4. *Podocarpus macrophyllus*; 5. *Nageia nagi*.

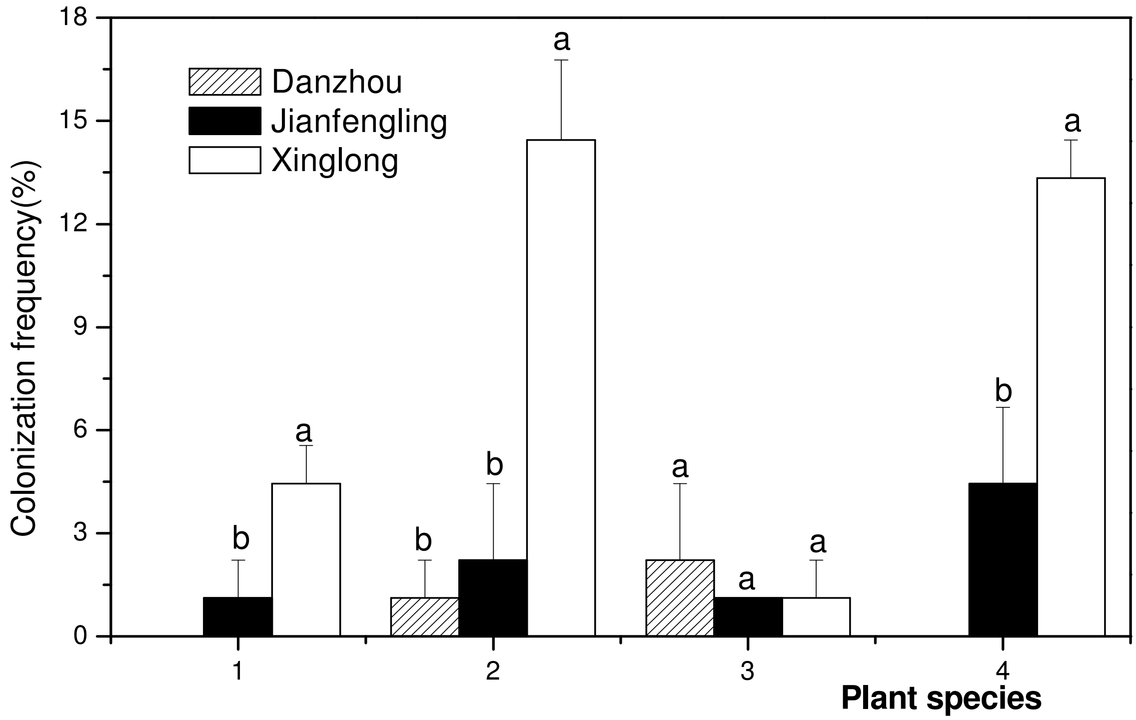

Figure 3. Colonization frequencies of endophytic *Pestalotiopsis* in the leaves of Palmae in three locations. 1. *Hyophorbe lagenicaulis*; 2. *Wodyetia bifurcate*; 3. *Caryota ochlandra*; 4. *Chrysalidocarpus lutesens*.

Chrysalidocarpus lutesens in 2008. The analysis of the colonization frequencies of endophytic *Pestalotiopsis* in five plants of Podocarpaceae between 2007 and 2008 revealed that the colonization frequencies of endophytic *Pestalotiopsis* in 2007 were notably higher than that in 2008, whereas no marked difference in colonization frequencies of *P. macrophyllus* was observed between 2007 and 2008 (Figure 2).

Colonization frequencies of endophytic *Pestalotiopsis* in different locations

Four plants of Palmae were selected to study the influence of sampling site on colonization frequencies of endophytic *Pestalotiopsis* in Danzhou, Jianfengling and Xinglong (Figure 3). For colonization frequencies on different sites, no significant difference for *C. ochlandra* were found, however the colonization frequencies of *C. lutesens* showed distinctly difference in the three locations. Moreover, the colonization frequencies in *H. lagenicaulis*, *Wodyetia bifurcata* and *C. lutesens* in Xinglong were higher than those for the other two positions. It was obviously interesting that the colonization frequencies of endophytic *Pestalotiopsis* of some plants showed significant difference in different sites, but some plants showed no distinct difference. It indicated that the colonization frequencies of endophytic *Pestalotiopsis* varied as sampling positions and plant species varied.

Colonization frequencies of endophytic *Pestalotiopsis* in different tissue

Nine mangrove plants and five Planchonellae plants were selected to study the influence of different tissues on colonization frequencies of endophytic *Pestalotiopsis* (Figure 4).

The colonization frequencies of endophytic *Pestalotiopsis* species in twigs were significant higher than that in leaves ($P<0.05$, Figure 4), especially, when there was no endophytic *Pestalotiopsis* isolated from the leaves in some host plants (*Kandelia candel*, *Bruguiera sexangula*, *Rhizophora mucronata*, *Aegiceras coniculatum*, *Madhuca hainanensis*, *Mimusops elengi*, *Synsepalum dulcificum* and *Lucuma nervosa*).

Species composition of endophytic *Pestalotiopsis* species in different host plants

Species composition of endophytic *Pestalotiopsis* varied in different families of plants (Table 1). Among 51 endophytic *Pestalotiopsis* species, 23, 11, 9 and 8 species were obtained from the families of Palmae,

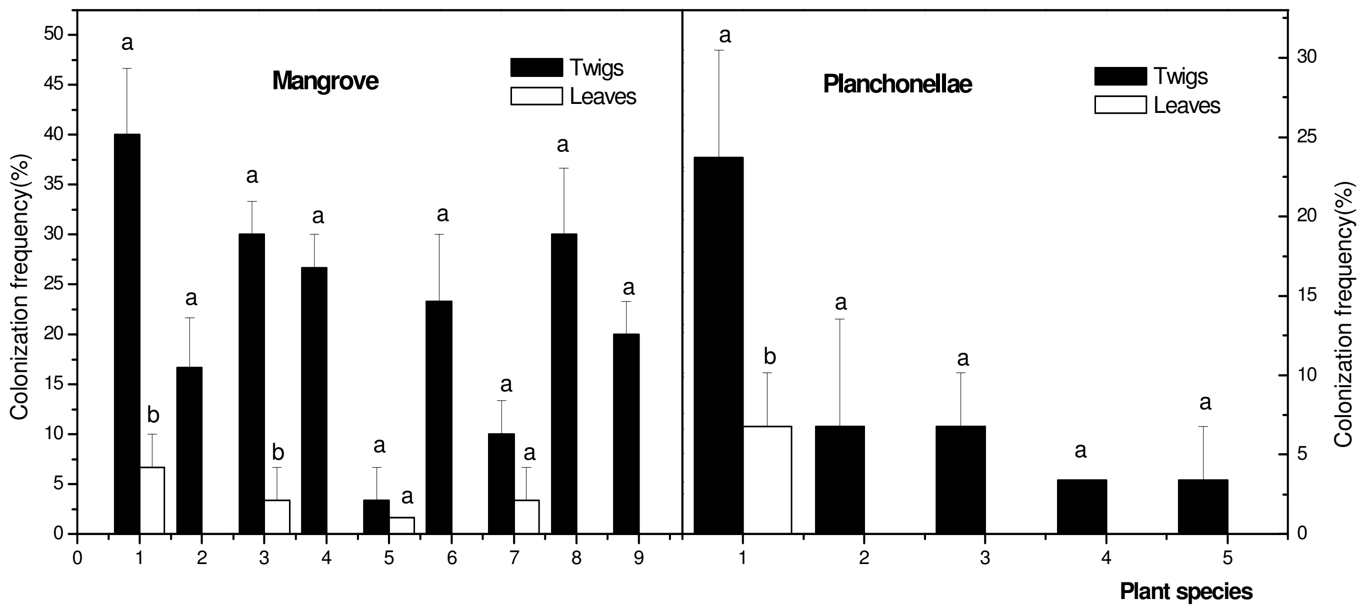

Figure 4. Colonization frequencies of endophytic *Pestalotiopsis* in different organization. Mangrove: **1**. *Bruguiera gymnorrhiza*; **2**. *Kandelia candel*; **3.** *Bruguiera sexangula*; **4**. *Bruguiera sexangula*; **5**. *Ceriops tagal*; **6**. *Rhizophora mucronata*; **7**. *Hibiscus tiliaceus*; **8**. *Aegiceras coniculatum*; **9**. *Xylocarpus granatum*. Planchonellae: **1**. *Manilkara zapota*; **2**. *Madhuca hainanensis*; **3**. *Mimusops elengi*; **4**. *Synsepalum dulcificum*; **5**. *Lucuma nervosa*.

Rhizophoraceae, Podocarpaceae and Planchonellae, respectively. Species composition of endophytic *Pestalotiopsis* also displayed great differences in different plants of same family. Among 16 palmaceous plants, 8, 7, 5 and 5 species were isolated from *Roystonea regia*, *Z. wallichiana, N. decaryi* and *C. lutesens*, respectively. However, for the other 12 plants, only 1 to 3 species were obtained from each plant. The average of the species number of endophytic *Pestalotiopsis* in palmaceous plants was 2.7. The endophytic *Pestalotiopsis* species number varied from 1 to 8, 1 to 3 and 1 to 5 in different mangrove plants, Podocarpaceae and Planchonellae, with an average of 3.0, 2.2 and 2.6 species per host, respectively.

A total of 180, 52, 59 and 38 *Pestalotiopsis* isolates were obtained from plants of Palmae, Rhizophoraceae, Podocarpaceae and Planchonellae, respectively. The most common species of endophytic *Pestalotiopsis* in plants of Hainan Province were *P. adusta*, *P. clavispora*, *P. paeoniae*, *P. virgatula* and *P. zonata* (Table 2). The most common *Pestalotiopsis* species were similar between Palmae (*P. clavispora*, *P. virgatula* and *P. zonata*) and Podocarpaceae (*P. virgatula* and *P. zonata*) and the most common *Pestalotiopsis* species in plants of Rhizophoraceae and Planchonellae were *P. adusta* and *P. paeoniae*, respectively.

Shannon-Wiener diversity index of endophytic *Pestalotiopsis* species in plants of both Palmae (2.4641) and Rhizophoraceae (2.5013) were much higher than that in Podocarpaceae (1.4775) and in Planchonellae (1.8707) (Table 2). Although, 52 strains were isolated from Rhizophoraceae, no more than three strains were isolated from Palmae. The Evenness index and Shannon-Wiener index of endophytic *Pestalotiopsis* were very high among the four families.

DISCUSSION

Colonization frequencies of endophytic *Pestalotiopsis*

Okane et al. (1998) found that endophytic *Pestalotiopsis* species were colonized in seven species of Ericaceae with different colonization frequencies (0.7 to 17.1%) in Kyoto and Japan. Cannon and Simmons (2002) reported the diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. In contrast to studies in temperate ecosystems, no distinct fungal communities were identified for individual plant species, suggesting that the degree of host preference was low. The results of this study showed that colonization frequencies of endophytic *Pestalotiopsis* in Hainan varied with different host plants, but the degrees of host preference were different in different plant families.

The colonization frequencies of endophytic *Pestalotiopsis* species varied with different host tissues in this study. Taylor et al. (1999) investigated the endophytes in *Arenga tremula* from four locations. Quantitative and qualitative differences in endophyte assemblages from old and young tissues were observed, and more isolates were recovered from old tissues

Table 1. Host plants and their endophytic *Pestalotiopsis* species in Hainan, China.

Host plants	*Pestalotiopsis* species
Palmae	
Chamaedorea elegans Mart. Liebm	*P. clavispora*
Areca catechu L.r	*P. photiniae*
Roystonea regia (H.B.K) Cook.	*P. briosiana*, *P. elastica*, *P. fuchsiae*, *P. photiniae*, *P. palmarum*, *P. pandani*, *P. virgatula*, *P. zonata*
Cyrtostachys renda Bl.	*P. cinchonae*
Wodyetia bifurcata A.K.Jrvine	*P. clavispora*, *P. menezesiana*
Chamaedorea oblongata Mart.	*P. briosiana*, *P. virgatula*, *P. zonata*
Hyophorbe lagenicaulis Mart.	*P. bicolor*, *P. clavispora*
Chamaedorea metallica O.F.Cook ex H.E.Moore	*P. clavispora*
Livistona chinensis(Jacq.)R.Br.	*P. foedans*
Neodypsis decaryi Jum	*P. clavispora*, *P. gracillis*, *P. lambertiae*, *P. pauciseta*, *P. zonata*
Chrysalidocarpus lutesens H. Wendl.	*P. adusta*, *P. clavispora*, *P. gracillis*, *P. virgatula*, *P. zahlbruckneriana*
Arenga engleri Beccari	*P. clavispora*
Zalacca wallichiana Salacca.	*P. adusta*, *P. algeriensis*, *P. briosiana*, *P. fici*, *P. palmarum*, *P. virgatula, P. zonata*
Cocos nucifera L.	*P. cinchonae*
Elaeis guineensis Jacq.	*P. heterocornis*
Caryota ochlandra Hance	*P. coffeae*, *P. peycnonelii*, *P. Sorbi*
Mangrove plants	
Bruguiera sexangula (Lour.) Poir.	*P. adusta*, *P. clavispora*, *P. foedans*, *P. Pauciseta*
Lagerstroe miaindica L.	*P. clavispora*
Rhizophora mucronata Lam.	*P. adusta P. Clavispora*
Hibiscus tiliaceus L.	*P. adusta*, *P. Clavispora*
Bruguiera sexangula Lour	*P. adusta*, *P. heterocornis*, *P. Clavispora*
Ceriops tagal (Perr.)C.B.Rob.	*P. adusta*
Xylocarpus granatum Koenig	*P. adusta*
Bruguiera gymnorrhiza (L.) Poir.	*P. adusta*, *P. clavispora*, *P. gracillis*, *P. heterocornis*, *P. neglecta*, *P. virgatula*
Kandelia candel L.	*P. adusta*, *P. cinchonae*, *P. gracillis*, *P. paeoniae*, *P. pauciseta*, *P. photiniae*, *P. vaccinii*, *P. virgatula*
Aegiceras coniculatum (L.) Blanco	*P. foedans*, *P. Virgatula*
Podocarpaceae	
Podocarpus fleuryi Hickel	*P. virgatula*
Podocarpus macrophyllus var. maki	*P. photiniae*, *P. virgatula*
Podocarpus imbricatus Bl.	*P. clavispora*, *P. versicolor*
Dacrydium pierrei Hichel	*P. alöes, P. vismiae*, *P. Zonata*
Podocarpus macrophyllus (Thunb.) D. Don.	*P. cinchonae*, *P. Hainanensis*
Nageia nagi (Thunb.) O. Ktze.	*P. clavispora*, *P. virgatula*, *P. Zonata*
Planchonellae	
Lucuma nervosa A.DC.	*P. alöes*, *P. clavispora*, *P. theae*, *P. virgatula*, *P. Zonata*
Madhuca hainanensis Chun et How	*P. paeoniae*
Manilkara zapota (Linn.) Van Royen	*P. leucothoes*, *P. paeoniae*, *P. subcuticulari*, *P. Zonata*
Synsepalum dulcificum Denill	*P. alöes*, *P. Theae*
Mimusops elengi Linn	*P. zonata*

Table 2. The composition of the endophytic *Pestalotiopsis* from the four families of the host plants.

Species	Number of the isolates/ Relative abundance (%)			
	Palmae	Rhizophoraceae	Podocarpaceae	Planchonellae
P. adusta	5/2.78	20/38.46	-	-
P. algeriensis	1/0.56	-	-	-
P. alöes	-	-	3/5.08	2/5.26
P. bicolor	2/1.11	-	-	-
P. briosiana	10/5.56	-	-	-
P. cinchonae	3/1.67	1/1.95	46.78	-
P. clavispora	33/18.33	9/17.31	14/16.95	7/18.42
P. coffeae	2/1.11	-	-	-
P. elastica	1/0.56	-	-	-
P. fici	2/1.11	-	-	-
P. foedans	2/1.11	2/3.85	-	-
P. fuchsiae	2/1.11	-	-	-
P. gracillis	21/11.67	5/9.62	-	-
P. hainanensis	-	-	1/1.69	-
P. heterocornis	3/1.67	3/5.77	-	-
P. lambertiae	1/0.56	-	-	-
P. leucothoes	-	-	-	3/7.89
P. menezesiana	16/8.89	-	-	-
P. neglecta	-	3/5.77	-	-
P. paeoniae	-	1/1.92	-	11/28.94
P. palmarum	3/1.67	-	-	-
P. pandani	1/0.56	-	-	-
P. pauciseta	2/1.11	3/4.77	-	-
P. peycnonelii	1/0.56	-	-	-
P. photiniae	8/4.44	1/1.92	3/5.08	-
P. sorbi	1/0.56	-	-	-
P. subcuticulari	-	-	-	3/7.89
P. theae	-	-	-	4/10.53
P. vaccinii	-	1/1.92	-	-
P. versicolor	-	-	3/5.08	-
P. virgatula	22/12.22	3/5.77	13/22.03	1/2.63
P. vismiae	-	-	1/1.69	-
P. zahlbruckneriana	1/0.56	-	-	-
P. zonata	37/20.56	-	21/35.59	7/18.42
Total number of isolates	180	52	59	38
Shannon-Wiener index (H')	2.4641	2.5013	1.4775	1.8707
Evenness index (J)	0.7859	1.0431	0.3624	0.8996
Richness index (S)	23	11	9	8

independent of the age of the palm. The distribution of endophytic fungi might be affected by tissue texture, physiology and chemistry (Petrini and Carroll, 1981; Polishook et al., 1996; Arnold et al., 2001; Cheng et al., 2009). In this study, the colonization frequencies of endophytic *Pestalotiopsis* were different in different tissue of same plant. In general, colonization frequencies in twigs were relatively higher than that in leaves.

The colonization frequencies of endophytic *Pestalotiopsis* in plant were influenced by the natural condition. Kumaresan and Suryanayanan (2001) investigated the endophytic fungi of *R. apiculata* and *R. mucronata* growing in the Pichavaram mangrove of Tamil Nadu, Southern India. The results showed that more endophytes could be isolated during the rainy months than the dry period. Similar result was obtained in other study (Maria et al., 2003; Karamchand et al., 2009). In this study, the colonization frequencies of endophytic *Pestalotiopsis* in plant samples of Palmae and Podocarpaceae collected in April 2007 were significantly higher than that collected in April 2008. It was rainless in most area of Hainan during September 2007 to May 2008. The drought and high temperature may be the reasons for the differences in 2007 and 2008.

Species composition of endophytic *Pestalotiopsis*

A total of 43 endophytic *Pestalotiopsis* species were isolated from 37 plant species belonging to 4 families. Species composition of endophytic *Pestalotiopsis* varied in the different families of plants even though, in same family, species composition of endophytic *Pestalotiopsis* also displayed great differences. The number of *Pestalotiopsis* species isolated from Palmae was highest in the four plant families. This was mainly because the numbers of plant species in Palmae which were selected for comparison with the other three families in this study were more. This is also the main reason while the Evenness and Shannon-Wiener indexes of endophytic *Pestalotiopsis* were the highest in Rhizophoraceae and not in Palmae.

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