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## Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*

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A total of 343 endophytic fungal isolates representing 60 taxa including 30 morphotypes were isolated from the different parts of the Chinese medicinal plant, *Tripterygium wilfordii*. In most cases fungal strains were only identified to genus because species identification was difficult in these speciose genera. Non-sporulating isolates were designated as Morphotypes 1 to 30. The endophytic assemblages of *T. wilfordii* comprised a number of cosmopolitan species such as *Colletotrichum gloeosporioides*, *Guignardia* sp., *Glomerella cingulata*, *Pestalotiopsis* spp., *Phomopsis* spp. and *Phyllosticta* sp. The overall fungal community of *T. wilfordii* was moderately diverse. The fungal community from the twig xylem parts was most diverse, followed by leaves, twig bark, root xylem and flowers. *Pestalotiopsis cruenta*, *Phomopsis* sp. B and *Phomopsis* sp. A were predominantly isolated from the twig xylem and bark. These endophytes were not isolated from the roots, leaves and flowers. Likewise, *Glomerella cingulata* and *Guignardia* sp. were predominantly isolated in leaves. *Phialophora* sp. was isolated only in root xylem. In contrast, *Pestalotiopsis disseminata* was isolated from all the tissues except root bark. Morphotype sp. 1 was isolated from twig and root segments. Interestingly, root bark only accommodated Morphotype sp. 1 and no other endophytic fungi were isolated from the organ. *Pestalotiopsis* spp. were frequently isolated as root endophytes in this study. The species composition and frequency of endophyte species was found to be dependent on the tissue type. The dominant fungi isolated from the different tissues of the host expressed a fair degree of tissue-recurrence.

**Key words:** Chinese medicinal plant, diversity, fungal distribution, novel drug production, tissue-recurrence, Traditional Chinese Medicine.

### Introduction

An estimated 70,000-100,000 fungal species have been identified which is *ca.* 5% of the total estimated 1.5 million fungi on this planet (Hawksworth, 1991, 2001). Dreyfuss and Chapela (1994) estimated that endophytic fungi from the 270,000 species of plants existing on this planet could account for

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$1.38 \times 10^6$  unique fungal species. The enormity of this estimation is due to the fact that fungal endophytes have been isolated from every plant species examined to date and endophytes are important components of fungal biodiversity (Arnold *et al.*, 2000; Kumaresan and Suryanarayanan, 2002).

Endophytic fungi are probably one of the major potential sources for new, useful metabolites (Dreyfuss and Chapela, 1994). There has been a great interest in endophytic fungi as potential producers of novel, biologically active products (Schulz *et al.*, 2002; Strobel and Daisy, 2003; Tomita, 2003; Urairuj *et al.*, 2003; Wildman, 2003). Some endophytic fungi from Chinese medicinal plants are also potential sources of diverse bioactive metabolites that may have potential for therapeutic purposes (Tan *et al.*, 2000; Tan and Zou, 2001). If these fungi could be utilized to produce the bioactive compounds of medicinal plants on large-scale fermentors, this would provide a new technology for producing many types of Traditional Chinese medicine. Some endophytic fungi have been found to produce similar medicinal compounds to that of the host. Proof of principal was realized when the anticancer drug taxol was found to be produced by endophytic fungi isolated from *Taxus brevifolia* (Strobel *et al.*, 1996). Screening of this diverse group of fungi that may produce valuable medicinal plant products is a promising approach for obtaining Traditional Chinese Medicine from plants on a commercial scale using microbes (Strobel, 2003; Strobel and Daisy, 2003).

*Tripterygium wilfordii* (Lei gong deng) is a perennial vine belonging in *Celastraceae*. The plant has a long history in Traditional Chinese Herbal Medicine for the treatment of fever, chills, edema (abnormally large fluid volume) and inflammation. *Tripterygium wilfordii* contains more than 70 components, including diterpene, sesquiterpenes, triterpenes, glycosides, dulcitol, wilfordine, quinone and alkaloids (Kutney *et al.*, 1992). The clinically active compound triptolide and its derivatives have been isolated from the all parts of *Tripterygium wilfordii* and the large-scale extraction is mainly from roots (Yang *et al.*, 2000). Previous studies concerning fungal endophytes from this plant have concentrated on the production of bioactive compounds (Lee *et al.*, 1995; Strobel *et al.*, 1999; Wagenaar and Clardy, 2001). We have screened an array of endophytic fungi from different parts of *Tripterygium wilfordii* for immunomodulatory activity (Kumar *et al.*, 2004). Extracted products of these fungi showed anti-proliferative activity against peripheral blood mononuclear cells (PBMC) with no cytotoxicity. Among them, purified compounds of *Pestalotiopsis* sp. (HKUCC 10197) exhibited significant inhibition on proliferation of PBMC, leading to a lower T-cell subpopulation, cytokine and immunoglobulin (IgG and IgM) production (Kumar *et al.*, 2003, 2004). We have not however, reported our results on the

biodiversity and tissue-recurrence of fungal endophytes from this Chinese medicinal plant. This present study was undertaken in order to investigate (1) the pattern of colonisation and distribution of fungal endophytes in different organs of *Tripterygium wilfordii*; (2) to study the diversity of fungal endophytes of *Tripterygium wilfordii*; and (3) to investigate the tissue-recurrence of fungal endophytes of *Tripterygium wilfordii*.

## **Materials and methods**

### ***Host and site selection***

*Tripterygium wilfordii* samples were collected from Xenzhen Forest Station, Weizhi county located in Guangdong Province (24° 17' 14" N and 112° 24' 55" E) in March 2001.

### ***Collection of Tripterygium wilfordii samples***

Leaves, stems, bark, roots and flowers of five *T. wilfordii* plants growing 50-100 m apart were chosen. Five leaves, five 10 cm long twigs and roots samples and five inflorescences were collected from each plant and placed in 'Snap-lock' plastic bags and returned to the laboratory on the same day and kept at 4°C until the next morning for the isolation of endophytes.

### ***Treatments of materials***

Endophytes were isolated from plant tissues using modifications of method of Kumaresan and Suryanarayanan (2002) following pilot experiments. The following protocol was adopted. Plant specimens were thoroughly washed in running tap water for 10 minutes. Four 6-mm diam. disks were cut from each leaf using a sterile cork borer. Twigs and roots were cut into 2 cm long segments. Each segment was debarked to separate bark from xylem. Flowers were treated as a separate entity and 20 individual flowers were taken from each inflorescence. All samples were surface sterilized by dipping in 75% ethanol for 1 minute, followed by a solution of Chlorox (3.25%) for 3 minutes, and finally 75% ethanol for 30 seconds. Surface sterilized samples were washed with three changes of sterile distilled water and blotted with sterile tissue paper and the effectiveness of sterilization was double checked by following the method of Schulz *et al.* (1993). The plant segments were then transferred to Potato Dextrose Agar (PDA, Oxoid) plates amended with 1%

streptomycin to inhibit bacterial growth. Plates were labelled accordingly and incubated at 24°C with a 12-hour cycle of dark and light (Lacap *et al.*, 2003).

### ***Culturing and subculturing***

The growing edges of colonies from the plant segments were transferred to PDA plates by hyphal tipping (Strobel *et al.*, 1996). Continuous transfer of fungi was carried out as new colonies continued to appear for up to two or three weeks. Plates were then incubated and periodically ascertained for purity by hyphal tipping. The fungal isolates were numbered and stored in sterile distilled water at 4°C and in liquid nitrogen at -196°C. The stored cultures were submitted to the Hong Kong University Culture collection (HKUCC).

### ***Induction of sporulation***

The cultures, which failed to sporulate within 2-4 months of incubation were designated as ‘mycelia sterilia’ and sorted to morphotypes according to cultural characteristics (Guo *et al.*, 1998; Lacap *et al.*, 2003). Three methods were adopted to induce sporulation. In the first method, isolates were subcultured along with sterilized host parts and incubated at 24°C with a cycle of 12 UV light and dark. In the second method, the cultured plates were incubated with gamma-irradiated leaves (purchased from Penn State University, USA), which commonly allow the formation of fungal-fruiting bodies that aid their identification (Strobel *et al.*, 1996). In the third method, isolates were cultured on nutrient deficient potato carrot agar.

### ***Identification***

Pure cultures were examined periodically for sporulation and identified. Fungal identification methods were based on the morphology of the fungal culture, the mechanism of spore production and characteristics of the spore by following the standard mycological manuals (e.g. Ellis, 1971; Sutton, 1980; Nag Raj, 1993).

### ***Statistical analysis***

#### **Overall colonisation and isolation rate**

Measurement of fungal occurrence was established by calculating the colonisation density, colonisation rates and isolation rates. The density of

colonisation was calculated as the percentage of segments infected by one or more isolate(s) from the total number of segments of each tissue plated following the method of Petrini and Fisher (1988).

$$\text{Colonisation rate} = \frac{\text{Total no. of leaf discs/sections in a sample yielding } \geq 1 \text{ isolates}}{\text{Total no. of leaf discs/sections in that sample}}$$

$$\text{Isolation rate} = \frac{\text{Total no. of isolates yielded by a given sample}}{\text{Total no. of leaf discs/sections in that sample}}$$

One-way ANOVA was performed to compare the isolation rates and colonisation rates of fungal endophytes from different parts of *T. wilfordii* because the sample sizes were equal and conformed to the equal variance and normality tests. The significant differences amongst means were further tested by the Student-Newman-Keulis (SNK) test to correct the multiplicity (Zar, 1999).

### Species diversity indices

Species diversity is calculated in terms of species richness and evenness. The most common and widely used diversity index is the Shannon-Wiener Index ( $H_S$ ). It is an information statistic that is independent of sample size, and estimates diversity from random samples (Poole, 1974) and it also serves as a relative index for  $H_S$ .

The Gleason index ( $H_G$ ) is sensitive to richness aspects of diversity and the Shannon index ( $H_S$ ) includes both evenness and richness aspects (Groth and Roelfs, 1987). Relative indices were calculated for Gleason ( $H_{GR}$ ) and Shannon ( $H_{SR}$ ) to evaluate the ratio of species richness over the evenness in order to display the extent of species richness of the fungal community. The Simpson index is sensitive to abundances of the 1 or 2 most common species of a community and can be regarded as a measure of dominance (Simpson, 1949). Therefore, Shannon and Simpson indices combine species richness and abundance into a single value (Groth and Roelfs, 1987). A measure of the equitability of abundance of species was estimated by using Pielou's evenness index ( $J$ ) (Pielou, 1966). Pielou's evenness index ( $J$ ) is also considered as the relative index  $H_{SR}$  or Shannon evenness index. The equitability of the individuals, i.e. even spread of individuals are assumed when the evenness index value approaches 1. When this diversity index is equal to 1 this implies complete evenness (i.e. equally distributed species in a fungal community) and when it is equal to 0 it implies complete unevenness (Pielou, 1966). The  $H_S$

values for the fungal communities from each of the host parts were compared using one-way ANOVA and a Student-Neumen-Keuls's (SNK) multiple comparison test was performed for the mean diversity index value of the fungal communities from the different host parts (Zar, 1999). The formulae for computation of diversity indices such as Shannon index, Gleason index and its relative index, Pielou's evenness index and Simpson dominance index are presented below.

[1] Gleason index ( $H_G$ ) =  $N_p - 1 / \ln N_i$

[2] Shannon index ( $H_S$ ) =  $-\sum_j (p_j \ln p_j)$ ,  $j = 1, \dots, N_p$ , where  $N_i$  is the total number of individuals,  $N_p$  is the number of species identified among these isolates, and  $p_j$  is the proportion of individuals in the  $j^{\text{th}}$  species

[3] Relative index for Gleason index ( $H_{GR}$ ) =  $H_G / H_{G_{\max}} = N_p - 1 / N_i - 1$

[4] Relative index for Shannon index ( $H_{SR}$ ) or Pielou's evenness ratio ( $J$ ) =  $H_S / H_{S_{\max}} = H_S / \ln N_i$ ; where  $H_{G_{\max}}$  and  $H_{S_{\max}}$  are the greatest possible values of  $H_G$  and  $H_S$  in a sample of  $N_i$  individuals. These maximal values are reached for  $N_p = N_i$  (hence,  $p_j = 1/N_i$ , for all  $j$ s), and equal  $(N_i - 1) / \ln N_i$  and  $\ln N_i$  respectively.

[5] Simpson dominance index ( $D$ ) =  $1 / \sum_j (p_j^2)$

### Similarity index

To describe the taxonomic affinity of endophytic mycota among the various parts of the *Tripterygium wilfordii*, a Jaccard's coefficient (JI), was used to measure the similarity between pairs of samples (Arnold *et al.*, 2000).

$$JI = a / (a+b+c)$$

where  $a$  represents the number of species occurring in both samples,  $b$  represents the number of species restricted to sample 1, and  $c$  represents the number of species restricted to sample 2. JI ranges from 0 (no taxa shared) 1 (all taxa shared).

### Correspondence analysis

Correspondence analysis was used to analyse the tissue unit and fungal species ordinations to verify the ecological interrelationships between tissue units and fungal species in a single analysis. JMP software was used to carry out the correspondence analysis.

## Results

### *Composition of endophytic fungi*

Table 1 shows the relative colonisation densities in percentages, total number of taxa and total number of isolates for each sample type. Totally 343 isolates were recovered from 500 samples (100 segments of stem bark, stem xylem, root bark, and 100 disks of leaves and 100 flowers) and among these isolates, 60 taxa were grouped according the morphological characters. Thirty taxa were identified to genus level that represents 263 (76.7%) identifiable isolates of the total isolates recovered. It does not include mycelia sterilia of which there were 80 isolates (23.6%). Fifteen taxa were present at relative colonisation densities > 5% in at least one type of tissue category.

There were 5 consistently recognizable mycelia sterilia (Morphotype species 1, 30, 31, 21 and 22) which comprised 52 isolates (15.6% of the total isolates) and 28 (8.2%) miscellaneous mycelia sterilia. The numbers of taxa in twig bark and root xylem was similar ranging between 13 and 14. About 55% of the species present have a colonisation density of between 2% and 40%. The remaining species occurred rarely, having colonisation densities of less than 1%.

### *Tissue-recurrence*

The fungal community of twig bark appeared to be dominated by *Pestalotiopsis cruenta*, *P. disseminata* and *Phomopsis* sp. B. Complete twig xylems were essentially dominated by *Phoma* sp. 1 (R.C.D = 22%) that formed fruiting bodies only on sterilized twig xylem tissue. *Acremonium* sp. A, *Pestalotiopsis cruenta* and *Phomopsis* sp. A were the next most dominant species infecting 10-13% R.C.D of twig xylem tissues.

Complete root bark were colonised by only one tan coloured Morphotype sp. 1 infecting 20% of segments. Root xylem was mainly colonised by *Phialophora* sp. (11%), which was virtually absent from other host organs. The tan coloured Morphotype sp. 1 (5%) was the second most dominant coloniser.

The endophytic population of leaves was dominated by *Guignardia* sp., *Phyllosticta* sp., *Glomerella cingulata* and *Colletotrichum gloeosporioides*, which occurred on more than 35% of the leaf disks. There are two probable anamorph-telomorph connections in leaf parts of this plant (*Glomerella cingulata* - *Colletotrichum gloeosporioides* and *Guignardia* sp. - *Phyllosticta* sp.). *Guignardia* sp. and *Phyllosticta* sp. were not identified to species level, however, both ascospores and conidia were identified from the same culture. Flowers were colonised mainly by *Phomopsis* sp. D (13%) and Morphotype sp. 30 (7%).

**Table 1.** Relative colonisation densities (% R.C.D.) of fungal endophytes isolated from different tissues of *Tripterygium wilfordii*. Fungal taxa are listed in ascending order of R.C.D.

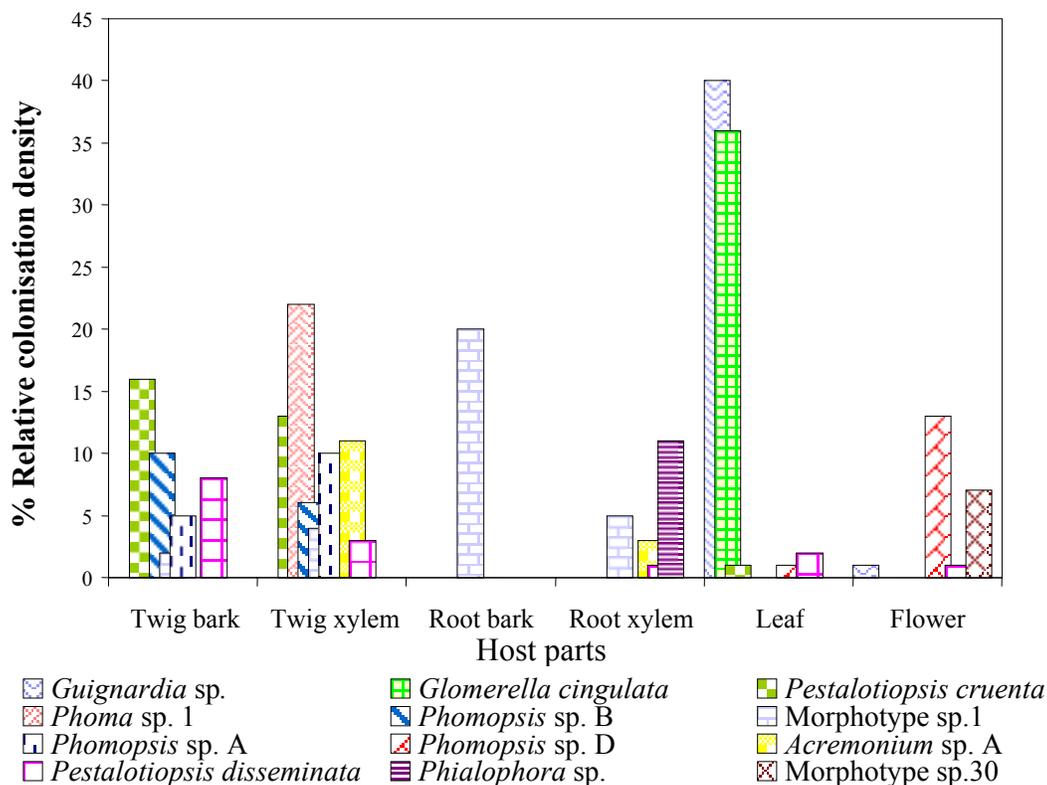
<b>Taxa</b>	<b>HKUCC no.</b>	<b>Twig bark</b>	<b>Twig xylem</b>	<b>Root bark</b>	<b>Root xylem</b>	<b>Leaves</b>	<b>Flowers</b>
<i>Guignardia</i> sp.	10141	-	-	-	-	40	1
<i>Glomerella cingulata</i>	10142	-	-	-	-	36	-
<i>Pestalotiopsis cruenta</i>	10186	16	13	-	-	1	-
Morphotype sp. 1	10143	2	4	20	5	-	-
<i>Phoma</i> sp. 1	10144	-	22	-	-	-	-
<i>Pestalotiopsis disseminata</i>	10187	8	3	-	1	2	3
<i>Phomopsis</i> sp. B	10188	10	6	-	-	-	-
<i>Phomopsis</i> sp. A	10145	5	10	-	-	-	-
<i>Phomopsis</i> sp. D	10146	-	-	-	-	1	13
<i>Acremonium</i> sp. A	10189	-	11	-	3	-	-
<i>Phialophora</i> sp.	10147	-	-	-	11	-	-
Morphotype sp. 30	10148	-	-	-	-	-	7
<i>Phomopsis</i> sp. E	10149	-	-	-	-	6	-
Morphotype sp. 31	10150	-	-	-	-	-	6
<i>Phomopsis</i> sp. F	10151	-	-	-	-	4	1
<i>Acremonium</i> sp. B	10152	-	1	-	-	4	-
<i>Pestalotiopsis vismiae</i>	10190	4	-	-	-	-	-
Morphotype sp. 21	10153	-	-	-	-	4	-
Morphotype sp. 22	10154	-	-	-	-	4	-
<i>Phomopsis</i> sp. C	10155	-	3	-	-	-	-
<i>Colletotrichum</i> sp.1	10156	2	2	-	-	-	-
<i>Alternaria alternata</i>	10157	-	-	-	-	2	-
<i>Acremonium</i> sp. C	10191	-	-	-	2	-	-
<i>Monodictys</i> sp.	10158	-	-	-	2	-	-
<i>Mucor</i> sp.	10192	-	2	-	-	-	-
<i>Verticillium</i> sp.	10193	-	2	-	-	-	-
Morphotype sp. 6	10159	-	2	-	-	-	-
Morphotype sp. 7	10160	-	2	-	-	-	-
Morphotype sp. 8	10161	2	-	-	-	-	-
Morphotype sp. 9	10194	-	2	-	-	-	-
Morphotype sp. 12	10162	-	-	-	2	-	-
Morphotype sp. 19	10163	-	2	-	-	-	-
<i>Colletotrichum musae</i>	10164	-	-	-	-	1	-
<i>Acremoniella</i> sp.	10195	-	-	-	1	-	-
<i>Aspergillus</i> sp.	10165	-	-	-	1	-	-
<i>Colletotrichum</i> sp.2	10196	-	1	-	-	-	-
<i>Phoma</i> sp. 2	10166	-	1	-	-	-	-
<i>Pestalotiopsis</i> sp.	10197	-	-	-	1	-	-
<i>Pestalotiopsis suffocata</i>	10198	1	-	-	-	-	-
Coelomycete sp. 1	10167	1	-	-	-	-	-
Coelomycete sp. 2	10168	1	-	-	-	-	-
Coelomycete sp. 3	10169	-	-	-	1	-	-
Coelomycete sp. 4	10170	-	-	-	-	-	1

**Table 1 continued.** Relative colonisation densities (% R.C.D) of fungal endophytes isolated from different tissues of *Tripterygium wilfordii*. Fungal taxa are listed in ascending order of R.C.D.

Taxa	HKUCC no.	Twig bark	Twig xylem	Root bark	Root xylem	Leaves	Flowers
Coelomycete sp. 5	10171	-	-	-	-	-	1
Morphotype sp. 4	10199	-	-	-	1	-	-
Morphotype sp. 5	10200	-	-	-	1	-	-
Morphotype sp. 10	10172	1	-	-	-	-	-
Morphotype sp. 11	10173	-	1	-	-	-	-
Morphotype sp. 13	10174	-	1	-	-	-	-
Morphotype sp. 14	10175	-	1	-	-	-	-
Morphotype sp. 15	10176	-	1	-	-	-	-
Morphotype sp. 16	10177	-	-	-	1	-	-
Morphotype sp. 17	10178	1	-	-	-	-	-
Morphotype sp. 20	10179	-	1	-	-	-	-
Morphotype sp. 23	10180	-	-	-	-	1	-
Morphotype sp. 24	10181	-	-	-	-	1	-
Morphotype sp. 26	10182	-	-	-	-	1	-
Morphotype sp. 27	10183	-	-	-	-	1	-
Morphotype sp. 28	10183	-	-	-	-	1	-
Morphotype sp. 29	10185	-	-	-	-	1	-
Total R.C.D		54	94	20	33	111	33
Total number of taxa		13	22	1	14	18	8
Total number of segments		100	100	100	100	100	100

Fig. 1 shows the dominant fungi which colonised more than 6% R.C.D in any tissue types. This Fig. 1 also describes the pattern of distribution of the endophytes within the different organs. *Guignardia* sp. and *Glomerella cingulata* were the most frequent colonisers of leaves but were virtually absent from other tissue parts, with the exception of *Guignardia* sp. which was present in the flowers with 1% colonisation. Twig xylem was extensively colonised by *Phoma* sp. 1 (22%) which was not encountered in other parts of the host.

*Phialophora* sp. (11%) was restricted to the root xylem only and could not be isolated from the other host parts. The tan coloured Morphotype sp. 1 was the main coloniser of the root bark with 20% colonisation density. However, this isolate was also isolated from twig parts and root xylem with comparatively lower colonisation density (2-5%) than in root bark. *Phomopsis* sp. D and *Pestalotiopsis disseminata* (Fig. 1) were the only taxa isolated from most parts of the plant, with the exception of the flowers. Similarly, tan coloured Morphotype sp. 1 was also isolated from twig and root bark with the exception of leaves and flowers. These two isolates appeared to be equally



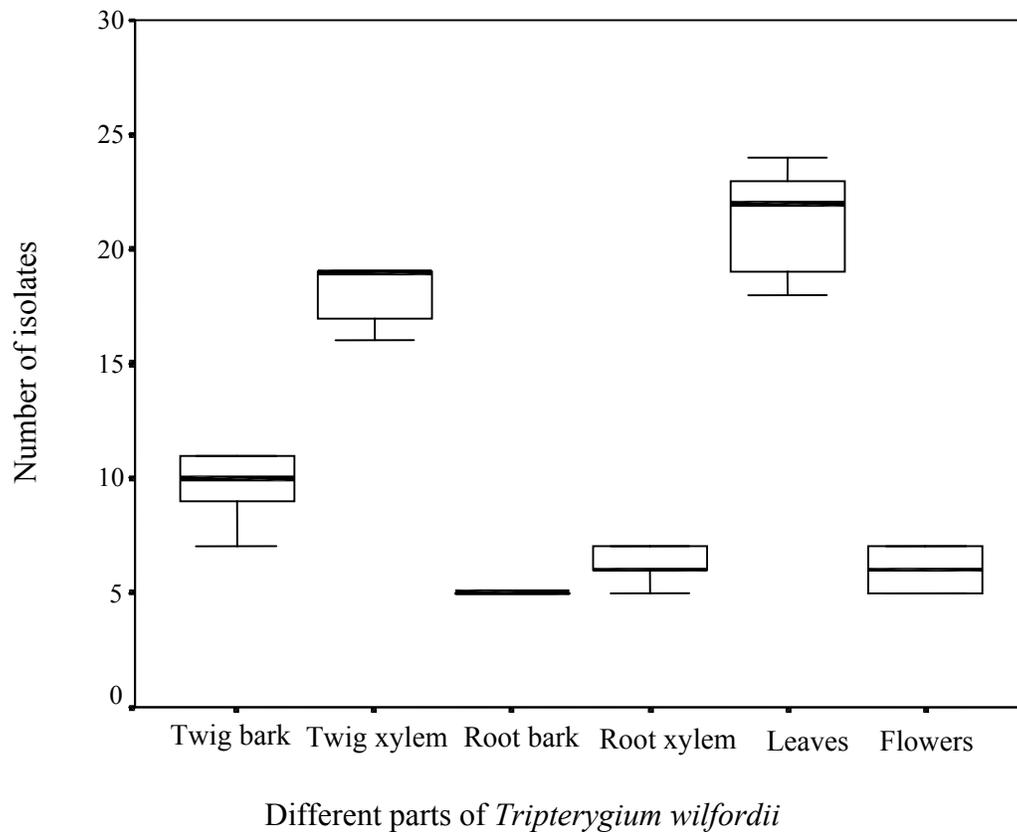
**Fig. 1.** Distribution of the predominant endophytic fungi from different host tissue.

distributed in the various host parts. The dominant fungi isolated from the different tissues of the host expressed a fair degree of tissue-recurrence.

***Colonisation and isolation rate from different tissues***

The mean overall colonisation and isolation rates of endophytes from *Tripterygium wilfordii* were 57.8% and 65.4% respectively (Table 2). The overall colonisation rate in the leaves was found to be significantly higher than those in root bark, root xylem, flowers and twig bark ( $p < 0.001$ ) with the exception of twig xylem ( $p = 0.107$ ). Similarly, overall colonisation rates of twig xylem were significantly higher than twig bark, root parts, leaf and flowers ( $p < 0.001$ ). Overall colonisation rates were highest in leaves, as reflected by the highest mean colonisation rate values (89%) followed by twig xylem (82%). The overall isolation rates from leaves were also significantly higher than those of twig xylem, twig bark, root bark, root xylem and flowers

and they were also significantly different when compared to other plant parts ( $p < 0.001$ ) including twig xylem ( $p < 0.05$ ). The overall isolation rate from the twig xylem was significantly higher than those of twig bark, root bark, root xylem and flowers ( $p < 0.001$ ) and the overall isolation rate of twig bark was significantly higher than those of root bark, flower and root xylem ( $p < 0.05$ ). The one-way ANOVA test reveals significant differences ( $p < 0.001$ ) in the mean values among the total number of isolates per tissue type. Therefore, it can be concluded that there are differences in the total number of isolates and their means from each tissue type. A box-plot was used to compare the number of isolates and their mean against tissue types (Fig. 2).



**Fig. 2.** Box-plots comparing the mean numbers of isolates recovered from twig bark; twig xylem, root bark, root xylem, leaves and flowers from five plants of *T. wilfordii*. Minimum and maximum values are indicated by the bars above the boxes (25-75%) with the mean value indicated by the inner black box.

**Table 2.** Overall colonisation and isolation rates of endophytic fungi from different organs of *Tripterygium wilfordii*.

	Twig bark	Twig xylem	Root bark	Root xylem	Leaves	Flowers	Whole plant
The mean overall colonisation rate (%)	46	82	20	28	89	27	57.8
The mean overall isolation rate (%)	48	94	20	32	100	31	65.4

**Table 3.** Species diversity in terms of richness, evenness and dominance of endophytic assemblages in different parts of *Tripterygium wilfordii*.

Fungal community	N <sub>i</sub>	N <sub>p</sub>	H <sub>G</sub>	H <sub>S</sub>	H <sub>SR</sub>	H <sub>GR</sub>	D
Twig bark	52	13	2.13 ± 0.25	1.64 ± 0.1	0.73 ± 0.04	0.56 ± 0.07	4.61 ± 0.30
Twig xylem	92	22	3.4 ± 0.55	2.23 ± 0.22	0.76 ± 0.05	0.55 ± 0.05	7.74 ± 1.5
Root bark	20	1	0	0	0	0	0
Root xylem	33	14	2.2 ± 0.36	1.57 ± 0.11	0.84 ± 0.12	0.78 ± 0.16	4.34 ± 0.97
Leaves	111	18	2.61 ± 0.42	1.74 ± 0.15	0.57 ± 0.03	0.39 ± 0.06	5.14 ± 0.45
Flowers	33	8	1.67 ± 0.45	1.26 ± 0.27	0.71 ± 0.12	0.66 ± 0.23	3.33 ± 0.89
Combined parts	343	60	6.26 ± 0.64	2.99 ± 0.10	0.72 ± 0.01	0.41 ± 0.02	15.5 ± 1.25

N<sub>i</sub> = Number of isolates, N<sub>p</sub> = Number of taxa, H<sub>G</sub> = Gleason index, H<sub>GR</sub>, H<sub>S</sub> = Shannon-Weiner index, H<sub>SR</sub> = Pielou's evenness ratio, D = Simpson dominance ratio. Each data represents the mean of five plants and the standard deviation (S.D).

### **Species diversity**

The diversity indices of the endophytic fungal communities from the various parts of the host are listed in Table 3. The Gleason index, Shannon index and Simpson dominance index values for the overall fungal community of *T. wilfordii* were 6.26, 2.99 and 15.5 respectively and had moderate diversity, probably due to the greater number of rare species present and low number of individuals.

The diversity (H<sub>S</sub>) of endophytes from the twig xylem appears to be higher than other host organs with a reasonable higher evenness index (J) followed by the leaf community. The very low evenness index value of the leaves as compared to other organs may be responsible for reducing its value of diversity index (H<sub>S</sub>) when compared with the diversity index of twig xylem. The low evenness values of leaves (H<sub>SR</sub> = 0.54) was due to the large number of individuals of *Guignardia* sp. and *Glomerella cingulata* isolated and lower number of isolates of other taxa in the leaf community. Similarly, evenness values for other tissues varied mainly due to the unequal distribution of

**Table 4.** Jaccard similarity index calculated for the fungal communities isolated from different parts of *Tripterygium wilfordii*.

	Twig bark	Twig xylem	Root bark	Root xylem	Leaves	Flowers
Twig bark	1.000					
Twig xylem	0.128	1.000				
Root bark	0.075	0.042	1.000			
Root xylem	0.074	0.079	0.067	1.000		
Leaves	0.054	0.063	0.000	0.028	1.000	
Flowers	0.050	0.033	0.000	0.048	0.147	1.000

respective individuals; therefore evenness accounts for rare species and measures the equitability between the species. The relative indices values for  $H_G$  ( $H_{GR}$ ) of all tissue parts were always lower than the relative indices for the  $H_S$  ( $H_{SR}$ ); inferring that species richness played a minor contribution to the overall diversity of the fungal community.

When one dominant species co-exists with a number of rare species, the condition is known as dominance. Simpson dominance index of the fungal community in the combined host parts ( $D = 15.5$ ) is higher than individual host parts. This is followed by that of twig xylem ( $D = 7.8$ ) and leaves ( $D = 5.92$ ) while the fungal community from twig bark and root xylem has similar dominance values ( $D = 4.3$ ) and the least dominance value was obtained from the fungal community from flowers ( $D = 3.5$ ). The highest dominance index value was in the combined host organs, twig xylems and leaves and is probably due to higher number of individual in some species.

A comparison of the Shannon diversity index ( $H_S$ ) for fungal communities of different plant parts showed the highest diversity in the twig xylem community ( $H_S = 2.23 \pm 0.22$ ), followed by that of leaves ( $H_S = 1.74 \pm 0.15$ ). There were significant differences in the diversity of fungal communities among different parts of *Tripterygium wilfordii* (one-way ANOVA,  $DF = 4$ ,  $20$ ,  $F = 16.342$ ,  $P < 0.001$ ). The mean diversity index from the one-way ANOVA was compared by SNK multiple comparison test. The mean diversity index of twig xylems and leaves were not significantly different ( $p > 0.05$ ), likewise the mean diversity index value of leaves and barks were not different ( $p > 0.05$ ). These values were, however, significantly different from the mean values for root and flowers ( $p < 0.05$ ).

### ***Similarity studies***

The comparison between the mycota recovered from the different host parts was computed using a Jaccard coefficient for possible pairs of host parts

(Table 4). The highest overlap (JI = 0.147) was observed for the fungal communities from leaves and flowers, followed by twig bark and twig xylem (JI = 0.128) indicating that the close proximity of tissue parts could share common fungi. The similarity between the endophytic assemblages of other parts of the host was quite low (JI = 0.028 to 0.079).

### ***Ordination***

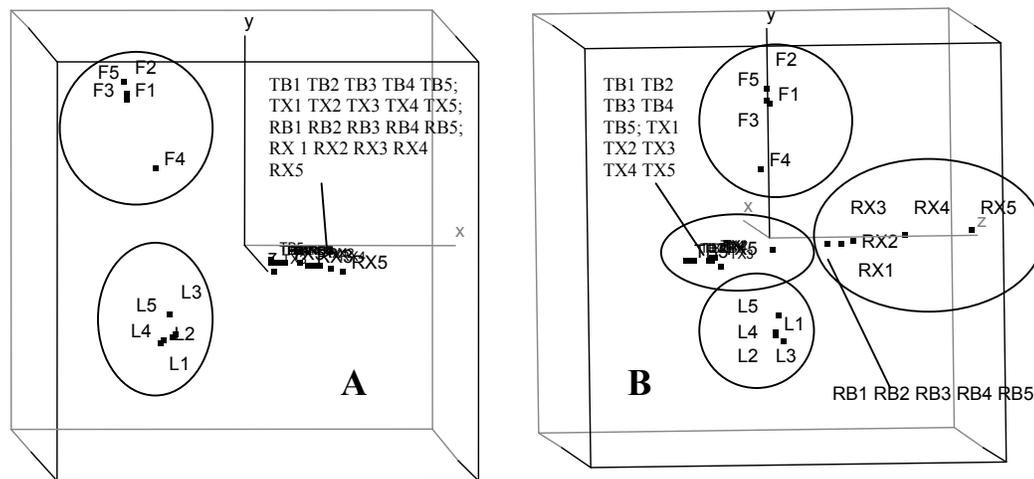
Ordination by 3-D correspondence analysis was performed to investigate patterns of endophyte assemblages on various tissue types of *Tripterygium wilfordii* (Fig. 3A,B). The first three principle axes of Fig. 3 explain 41.9% (x-axis: 15.1%, y-axis: 14.2%, z-axis: 12.6%) of the inertia or the variability in the data matrix. This is quite low indicating that the model does not correspond well with the data. However, when broadly interpreted, the analysis shows that the endophytic community from a given tissue taken from different specimens of *T. wilfordii* is quite similar, because clustering of samples is always consistent with the tissue from which they originate and it also shows clustering on basis of tissue-recurrence (Fig. 3). The x-axis clearly separates the fungal communities of twig from the other samples (Fig. 3A). The Y-axis on the other hand separates the fungal communities of flowers and leaves from other parts (Fig. 3A & B). Within this axis, there is a clear separation of fungal communities from flowers and leaves. The Z-axis separates the fungal communities of root bark and root xylem from other parts. The fungal communities (encircled in Fig. 3B) of twig and bark communities however, still cluster without separation.

### **Discussion**

#### ***Composition of endophytic community***

There have only been three reports of endophytes from *T. wilfordii* (Lee *et al.*, 1995; Strobel *et al.*, 1999; Wagenaar and Clardy, 2001), however, there is no data on the diversity of fungal endophyte communities associated with the host. The endophytes found in this study are therefore compared with the endophytes from other hosts from temperate and tropical regions. The endophyte mycota of *T. wilfordii* is moderately diverse (Table 1).

Sixty different fungal taxa were isolated from this plant. The number of taxa is higher than the 26 endophytic taxa from the Chinese medicinal plant *Brucea javanica* (Choi, 2002) but similar to 61 endophytic taxa from *Musa acuminata* (Photita *et al.*, 2001) and 63 endophytic taxa from palms



**Fig. 3.** Three-dimensional correspondence ordination of endophytic communities on various tissue parts of *Tripterygium wilfordii*. **A.** Diagram oriented at x- and y-axes. **B.** Diagram oriented at y- and z-axes. Tissue-types are indicated by: TB-twig bark, TX-twig xylem, RB-root bark, RX-root xylem, L-leaves. F-flowers. 1-5: number of trees.

(Rodrigues, 1994). The endophytic assemblages of *T. wilfordii* comprised a number of cosmopolitan species such as *Guignardia* sp., *Phyllosticta* sp., *Glomerella cingulata*, *Colletotrichum gloeosporioides*, *Phomopsis* spp. and *Pestalotiopsis* spp. All these genera have previously been isolated as endophytes from tropical regions and colonize leaves, bark, twigs or stems (Pettrini *et al.*, 1992; Taylor *et al.*, 1999; Fröhlich *et al.*, 2000; Photita *et al.*, 2001; Kumaresan and Suryanarayanan, 2002; Toofanee and Dulymamode, 2002). The endophytic flora of *Stylosanthes guianensis* is dominated mainly by *Glomerella cingulata*, *Xylaria* anamorphs and a *Phomopsis* sp. (Pereira *et al.*, 1993). Similarly, the endophytic mycota of *Brucea javanica* was also dominated by *Colletotrichum*, *Phomopsis* and *Xylariaceae* species (Choi, 2002). *Glomerella cingulata* and *Phomopsis* spp. were the dominant endophytes of *Tripterygium wilfordii* in this study. Most genera of fungal endophytes in this study were coelomycetes (35%) followed by mycelia sterilia (23.6%), hyphomycetes (15%) and zygomycetes (0.02%). The percentage of mycelia sterilia in this study was higher than the endophytes from tropical palm trees (e.g. *Licuala* sp. (12.9%) and *Trachycarpus fortunei* (11.2%) (Taylor *et al.*, 1999; Fröhlich *et al.*, 2000), however, the percentage was lower than the coastal redwood trees (26.9%) (Espinosa-Garcia and Langenheim, 1990). The overall composition of the endophytic communities of the six tissue parts was quite different.

### ***Colonisation and isolation rates***

The mean overall colonisation and isolation rates of endophytes from *Tripterygium wilfordii* were 48.3% and 66.7%, respectively, which is lower than that in *Brucea javanica* (Choi, 2002). Colonisation and isolation rates reported in *Brucea javanica* were 58.05% and 102.45%. The overall colonisation rate of the host plant was similar to the overall colonisation rate of *Musa acuminata* tissues, which were 48.9, 48, and 47.9 at different sites in Thailand (Photita *et al.*, 2001).

The overall colonisation (45-50%) and isolation rates (59-70%) in different plant individuals do not show much variation in contrast to the study of *Brucea javanica*, which showed great variation in the colonisation (6.25-100%) and isolation rates (31.25%-265.5%). This variation may be due to the difference in the number of collections made in these studies. Various host parts differed significantly in the number of endophytes. However, differences among tissue parts in number of isolates might not be biologically significant, despite being statistically significant.

### ***Distribution of dominant endophytes and their tissue recurrence***

The endophyte assemblage of the different tissue types showed that several endophytic fungi were common in more than one tissue (Table 1). Some of the dominant endophytes were isolated from almost every part of the tissues, for example *Pestalotiopsis disseminata* was isolated from every tissue part with exception of root bark (Fig. 1). Similarly, the tan coloured Morphotype sp.1 occupied twig bark and xylem and root bark and xylem (Fig. 1). In comparing endophytes from twig bark and twig xylem, *Pestalotiopsis cruenta*, *P. disseminata*, *Phomopsis* sp. B, *Phomopsis* sp. A and tan coloured Morphotype sp. 1 are common isolates from these tissues (Fig.1). The similarity index value also reflects this situation by indicating second highest overlap of species (JI = 0.128) (Table 4). This pattern indicates the ability of endophytes to penetrate from one part to another part of the host tissues. Bettuci and Saravay (1993) also reported that five endophytes were commonly isolated from the xylem and whole stem of *Eucalyptus globulus* and Fisher *et al.* (1995) reported that seven endophytes were commonly isolated from twig bark and twig xylem of *Gynoxis oleifolia*. Colonisation densities of *Pestalotiopsis cruenta*, *P. disseminata* and *Phomopsis* sp. B were consistently higher for bark than for xylem and other tissues. These data indicate that these taxa may be better adapted to living within superficial tissues as well as being able to colonize other tissues. This was pointed out for several bark and twig

endophytes in other hosts (Fisher and Petrini, 1987; Petrini and Fisher, 1988; Bettucci and Saravay, 1993; Fisher *et al.*, 1995). These studies also found a higher number of isolates in the bark than in the xylem tissues. The total colonisation density of bark (52%) was approximately half that of the xylem (92%) (Table 2). *Phoma* sp. (22%) was isolated only in twig xylem and had higher colonisation densities than other fungal isolates from this tissue. This taxon usually inhabits the xylem, as pointed out in endophyte tissue specificity studies in *Ulex europaeus* (Fisher and Petrini, 1987). However, several species of *Phoma* have commonly been isolated as endophytes from other organs of wide range of hosts (Petrini *et al.*, 1982; Rodrigues and Samuels, 1990; Fisher *et al.*, 1986; Bissegger and Sieber, 1994; Gindrat and Pezet, 1994; Sieber and Dorworth, 1994).

The root bark of *T. wilfordii* only harboured tan coloured Morphotype sp. 1 (20%) illustrating possible tissue-specificity or -recurrence in endophytic fungi. The low number of isolations of Morphotype sp. 1 and the absence of common endophytic fungi in the root bark may be due to the presence of a highly toxic insecticide wilfordine in root bark of the host plant. The root xylem was colonised by *Phialophora* (22%), which was not isolated from other tissues and also appears to be tissue-recurrent. A number of studies have indicated that *Phialophora* species are common root endophytes (Wang and Wilcox, 1985; Jumpponen and Trappe, 1988; Schulz *et al.*, 1999; Suryanarayanan and Vijaykrishna, 2001). Endophytes in aerial roots of *Ficus benghalensis* (Suryanarayanan and Vijaykrishna, 2001) are compared with this study because this plant is from a tropical region. The colonisation rate of *Phialophora* sp. (0.3%) was comparatively lower than in this study. Suryanarayanan and Vijaykrishna (2001) also encountered numerous soil fungi such as *Aspergillus* spp., *Gliocladium* spp., *Paecilomyces* sp. and *Penicillium* spp. In contrast, only *Aspergillus* sp. (1%) was isolated in this study. *Pestalotiopsis* spp. (1%) have been isolated for the first time as root endophytes, as species in the genus are usually isolated as foliar endophytes and pathogens.

Leaf disks were mainly colonised by *Glomerella cingulata* (40%) and *Guignardia* sp. (36%) followed by *Phomopsis* sp. E (Table 1). Some genera such as *Acremonium*, *Guignardia* and *Phomopsis* and some species, such as *Glomerella cingulata*, *Pestalotiopsis disseminata* obtained from the leaves in this study, were also isolated as endophytes from *Trachycarpus fortunei* in China (Taylor *et al.*, 1999). Sterile forms have often been isolated as leaf endophytes from many plants (Rajagopal and Suryanarayanan, 2000). In their study, they consistently isolated four sterile mycelia and *Fusarium avenaceum* only from the leaves of Indian medicinal plant, *Azadirachta indica*. In this

study, six sterile forms were isolated from the leaf segments and differentiated according to their morphology (Lacap *et al.*, 2003).

There has been no study of endophytes from flowers. A comparison between the endophytes of leaf and flower parts reveals there are possibly five common fungal taxa, i.e. *Guignardia* sp., *Phyllosticta* sp., *Phomopsis* sp. D, *Phomopsis* sp. E and *Pestalotiopsis disseminata* (Table 1).

Analysis of endophytic species composition and colonisation densities in twig bark, twig xylem, root bark, root xylem, leaves and flowers are characterized by different endophytic populations. The species composition and frequency of endophyte species is dependent on the tissue type. There were significant differences between the endophytes colonizing different tissues, and the mean overall colonisation rates and isolation rates of the leaf samples were higher than those of twig, root and flower samples (Table 2). The results are in agreement with previous studies indicating possible tissue-specificity or -recurrence in endophytes (Petrini and Fisher, 1988; Fisher and Petrini, 1990, 1992; Fisher *et al.*, 1991, 1995; Bettucci and Saravay, 1993; Frohlich *et al.*, 2000).

In the ordination analysis, the endophytes from the twig barks and twig xylem cluster together indicating some similarity in their endophyte communities. The leaf endophytes clustered separately, due to the presence of two dominant species, i.e. *Glomerella cingulata* and *Guignardia* sp. The separation of root communities is dependent on the presence, exclusively in the roots, of *Phialophora* sp., *Monodictys* sp. and *Acremonium* sp. C. The flower communities are clearly separate from the twig and root communities (Fig. 3A, B).

The overall tissue-recurrence is mainly based on the species composition and frequency that are known to vary with different tissues of the hosts (Rodrigues, 1994, 1996; Suryanarayanan and Vijaykrishna, 2001). Based on this concept, Petrini *et al.* (1992) stated that plant organs resemble distinct microhabitats with reference to endophyte infections. The outcomes of this study are in agreement with these observations.

### ***Diversity***

Endophytic fungal diversity is measured in terms of Shannon, Gleason, Simpson and Pielou's evenness indices (Table 3). The fungal community of *T. wilfordii* is moderately diverse. Most studies on endophyte communities discuss abundance and frequency. There have been only three studies of species diversity, evenness and dominance of endophytic communities (Espinosa-Garcia and Langenheim, 1990; Choi, 2002; Minguel and Bayman,

2003). The diversity ( $H_S = 3.11$ ) and equitability ( $J = 0.53$ ) index values of *Brucea javanica* in different collections could be comparable with the indexes of endophytes from the combined parts of *T. wilfordii* because *Brucea javanica* is a Chinese medicinal plant from China and was surveyed for endophytic fungi (Choi, 2002). In this study, however, the Simpson dominance index ( $D = 17.4$ ) value of *T. wilfordii* is remarkably higher than *Brucea javanica* ( $D = 4.13$ ). This is due to the presence of more dominant taxa in this study. Differences in endophytic communities in various parts of *T. wilfordii* were analysed in terms of diversity, evenness, species richness and dominance. Moderate species diversity and evenness were recorded in the overall combined host parts (Table 3). Values for the Gleason index were found to be agreement with those of Shannon index. The Shannon index values for the various tissue parts differed significantly (Table 3). The high species diversity and dominance were recorded in twig xylem when compared among the various tissue parts, followed by leaves. The evenness ( $H_{SR}$ ) has a minor contribution due to the unequal distribution of species in various tissue parts. Usually fungal diversity and abundance in xylem parts is lower than the bark, shoot and foliar parts (Stone *et al.*, 2000). In contrast, this study shows high diversity and abundance of endophytes in xylem compared to the other parts of the host. Given the small number of plants sampled (5 trees) and high variability within individual plants, it is hard to estimate the total endophytic diversity of each individual.

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### References

- Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. and Kursar, T.A. (2000). Are tropical fungal endophytes hyperdiverse? *Ecology Letters* 3: 267-274.
- Bettucci, L. and Saravay, M. (1993). Endophytic fungi of *Eucalyptus globulus*: a preliminary study. *Mycological Research* 97: 679-682.
- Bissenger, M. and Sieber, T.N. (1994). Assemblages of endophytic fungi in coppice shoots of *Castanea sativa*. *Mycologia* 86: 648-655.
- Choi, Y.W. (2002). *Biodiversity of Endophytic Fungi of Brucea javanica*. M.Phil. thesis. The University of Hong Kong.
- Dreyfuss, M.M. and Chapela, I.H. (1994). Potential of fungi in discovery of novel low molecular weight pharmaceuticals. In: *The discovery of Natural Products with Therapeutic Potential* (ed. V.P. Gullo). Butterworth-Heinemann, London, UK: 49-80.

- Ellis, M.B. (1971). *Dematiaceous Hyphomycetes*. CAB International, Oxon, UK.
- Espinosa-Garcia, F.J. and Langenheim, J.H. (1990). The endophytic fungal community in leaves of a coastal redwood population-diversity and spatial patterns. *New Phytologist* 116: 89-97.
- Fisher, P.J., Anson, A.E. and Petrini, O. (1986). Fungal endophytes in *Ulex europaeus* and *Ulex galli*. *Transactions of the British Mycological Society* 86: 153-193.
- Fisher, P.J. and Petrini, O. (1987). Tissue specificity by fungi endophytic in *Ulex europaeus*. *Sydowia* 40: 46-50.
- Fisher, P.J. and Petrini, O. (1990). A comparative study of fungal endophytes in xylem and bark of *Alnus* species in England and Switzerland. *Mycological Research* 94: 313-319.
- Fisher, P.J. and Petrini, O. (1992). Fungal saprobe and pathogens as endophytes of rice (*Oryza sativa* L.). *New Phytologist* 122: 137-143.
- Fisher, P.J., Petrini, L.E., Sutton, B.C. and Petrini, O. (1995). A study of fungal endophytes in leaves, stems and roots of *Gynoxis oleifolia* Muchler (Compositae) from Ecuador. *Nova Hedwigia* 60: 589-594.
- Fisher, P.J. and Petrini, O. and Webster, J. (1991). Aquatic hyphomycetes and other fungi in living aquatic and terrestrial roots of *Alnus glutinosa*. *Mycological Research* 95: 543-547.
- Fröhlich, J., Hyde, K.D. and Petrini, O. (2000). Endophytic fungi associated with palms. *Mycological Research* 104: 1202-1212.
- Gindrat, D. and Pezet, R. (1994). Le paraquat, un outil pour la révélation rapide d'infection fongiques latentes et de champignons endophytes. *Journal of Phytopathology* 141: 86-98.
- Groth, J.V. and Roelfs, A.P. (1987). The concept of measurement of phenotypic diversity in *Puccinia graminis* on wheat. *Phytopathology* 77: 1394-1399.
- Guo, L.D., Hyde, K.D. and Liew, E.C.Y. (1998). A method to promote sporulation in palm endophytic fungi. *Fungal Diversity* 1: 109-113.
- Hawksworth, D.L. (1991). The fungal dimension of biodiversity: magnitude, significance and conservation. *Mycological Research* 95: 641-655.
- Hawksworth, D.L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* 105: 1422-1431.
- Jumpponen, A. and Trappe, J.M. (1998). Dark septate endophytes: a review of facultative biotrophic root-colonising fungi. *New Phytologist* 140: 295-310.
- Kumar, S.S.D., Cheung, H.Y., Zhu, G.Y., Yang, D., Fong, W.F. and Hyde, K.D. (2004). Isolation and identification of triptonide and its analogus compounds from a fungal culture of *Pestalotiopsis leucothēs*. *Hong Kong Pharmaceutical Journal* 12: 158-164.
- Kumar, S.S.D., Lau, C.S., Chan, W.K., Yang, D., Cheung, H.Y., Chen, F. and Hyde, K.D. (2004). In vitro studies of endophytic fungi from *Tripterygium wilfordii* with anti-proliferative activity on human peripheral blood mononuclear cells. *Journal of Ethnopharmacology* (in press).
- Kumaresan, V. and Suryanarayanan, T.S. (2002). Endophytes assemblages in young mature and senescent leaves of *Rhizophora apiculata*: evidence for the role of endophytes in mangrove litter degradation. *Fungal Diversity* 9: 81-91.
- Kutney, J.P., Hewitt, G.M., Lee, G., Piotrowska, K., Roberts, M. and Rettig, S.J. (1992). Studies with the tissue cultures of the Chinese herbal plant, *Tripterygium wilfordii*, Isolation of metabolites of interest in rheumatoid arthritis, immunosuppression and male contraceptive activity. *Canadian Journal of Chemistry* 70: 1455-1480.
- Lacap, D.C., Hyde, K.D. and Liew, E.C.Y. (2003). An evaluation of the fungal 'morphotype' concept based on ribosomal DNA sequences. *Fungal Diversity* 12: 53-66.

- Lee, J.C., Lobokovsky, N.B., Pliam, N.B., Strobel, G.A. and Clardy, J.C. (1995). Subglutinol A and B: immunosuppressive compounds from the endophytic fungus *Fusarium subglutinans*. *Journal of Organic Chemistry* 60: 7076-7077.
- Minguel, A.G. and Bayman, P. (2003). Communities of endophytic fungi in leaves of tropical timber tree (*Guarea guidonia*: Meliaceae). *Biotropica* 33: 352-360.
- Nag Raj (1993). *Coelomycetous Anamorphs with Appendage Bearing Conidia*. Edwards Brothers Publishing Co., Ann Arbor, Michigan, USA.
- Pereira, J.O., Azevedo, J.L. and Petrini, O. (1993). Endophytic fungi of *Stylosanthes*: A first report. *Mycologia* 85: 362-364.
- Petrini, O. and Fisher, P.J. (1988). A comparative study of fungal endophytes in xylem and whole stems of *Pinus sylvestris* and *Fagus sylvatica*. *Transactions of the British Mycological Society* 91: 233-238.
- Petrini, O., Sieber, T.N., Toti, L. and Viret, O. (1992). Ecology, metabolite production and substrate utilization in endophytic fungi. *Natural Toxins* 1: 185-196.
- Petrini, O., Stone, J. and Carroll, F.E. (1982). Endophytic fungi in evergreen shrubs in western Oregon: a preliminary study. *Canadian Journal of Botany* 60: 789-796.
- Photita, W., Lumyong, S., Lumyong, P. and Hyde, K.D. (2001). Endophytic fungi of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park, Thailand. *Mycological Research* 105: 1508-1513.
- Pielou, E.C. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology* 13: 131-144.
- Poole, R.W. (1974). *An Introduction to Quantitative Ecology*. McGraw-Hill, New York.
- Rajagopal, K. and Suryanarayanan, T.S. (2000). Isolation of endophytic fungi from leaves of neem (*Azadirachta indica* A. Juss). *Current Science* 78: 1375-1378.
- Rodrigues, K.F. (1994). The foliar endophytes of the Amazonian palm *Eutrepe oleracea*. *Mycologia* 86: 376-385.
- Rodrigues, K.F. (1996). Fungal endophytes of palms. In: *Endophytic fungi in grasses and woody plants: Systematics, Ecology and Evolution* (eds. S.C. Redlin and L.M. Carris). APS Press, St. Paul, Minnesota, USA: 121-132.
- Rodrigues, K.F. and Samuels, G.J. (1990). Preliminary study of endophytic fungi in a tropical palm. *Mycological Research* 94: 827-830.
- Schulz, B., Boyle, C., Draeger, S., Römmert, A.K. and Krohn, K. (2002). Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycological Research* 106: 996-1004.
- Schulz, B., Römmert, A.K., Dammann, U., Aust, H.J. and Strack, D. (1999). The endophyte-host interactions: a balanced antagonism? *Mycological Research* 103: 1275-1283.
- Schulz, B., Wanke, U., Drager, S. and Aust, H.J. (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research* 97: 1447-1450.
- Sieber, T.N. and Dorworth, C.E. (1994). An ecological study about assemblages of endophytic fungi in *Acer macrophyllum* in British Columbia: in search of candidate mycoherbicides. *Canadian Journal of Botany* 72: 1397-1402.
- Simpson, E.H. (1949). Measurement of diversity. *Nature* 163: 688.
- Stone, J.K., Bacon, C.W. and White, J.F. (2000). An overview of endophytic fungi microbes: endophytism defined. In: *Microbial Endophytes* (eds. C.W. Bacon and J.F. White) Marcel Dekker, New York: 3-29.
- Strobel, G.A. (2003). Endophytes are sources of bioactive products. *Microbes and Infection* 5: 535-544.

- Strobel, G.A. and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews* 67: 491-502.
- Strobel, G.A., Hess, W.M., Ford, E., Sidhu, R.S. and Yang, X. (1996). Taxol from fungal endophyte and issue of biodiversity. *Journal of Industrial Microbiology* 17: 417-423.
- Strobel, G.A., Miller R.V., Miller, M.C., Condron, M.M., Teplow, D.B. and Hess, W.M. (1999). Crytocandin, a potent antimycotic from the endophytic fungus *Cryptosporipsis* cf. *quercina*. *Microbiology* 145: 1919-1926.
- Suryanarayanan, T.S. and Vijaykrishna, D. (2001). Fungal endophytes of aerial roots of *Ficus benghalensis*. *Fungal Diversity* 8: 155-161.
- Sutton, B.C. (1980). *The Coelomycetes - Fungi Imperfecti with pycnidia, acervuli and stromata*. Commonwealth Mycological Institute, Kew, UK.
- Tan R.X., Meng, J.C. and Hostettmann, K. (2000). Phytochemical investigation of some traditional Chinese medicines and endophyte cultures. *Pharmaceutical Biology* 38: 22-32.
- Tan, R.X. and Zou, W.X. (2001). Endophytes: a rich source of functional metabolites. *Natural Product Reports* 18: 448-459.
- Taylor, J.E., Hyde, K.D. and Jones, E.B.G. (1999). Endophytic fungi associated with the temperate palm, *Trachycarpus fortunei*, within and outside its natural geographic range. *New Phytologist* 142: 335-346.
- Tomita, F. (2003). Endophytes in Southeast Asia and Japan: their taxonomic diversity and potential applications. *Fungal Diversity* 14: 187-204.
- Toofanee, S.B. and Dulyamode, R. (2002). Fungal endophytes associated with *Cordemoya integrifolia*. *Fungal Diversity* 11: 169-175.
- Urairuj, C., Khanongnuch, C. and Lumyong, S. (2003). Ligninolytic enzymes from tropical endophytic Xylariaceae. *Fungal Diversity* 13: 209-219.
- Wagenaar, M.M. and Clardy, J. (2001). Dicerandols, new antibiotic and cytotoxic dimers produced by the fungus *Phomopsis longicolla* isolated from endangered mint. *Journal of Natural Products* 64: 1006-1009.
- Wang, C.J.K. and Wilcox, H.E. (1985). New species of ectendomycorrhizal and pseudomycorrhizal fungi: *Phialocephala finlandia*, *Chloridium paucisporum* and *Phialocephala fortinii*. *Mycologia* 77: 951-958.
- Wildman, H.G. (2003). The rise and fall of natural products screening for drug discovery. *Fungal Diversity* 13: 221-231.
- Yang, C.X., Zhou, T.C. and Qin, W.Z. (2000). Dynamic assay of triptolide in *Tripterium wilfordii*. *Shanghai Hospital Pharmacy* 11: 1-4.
- Zar, J.H. (1999). *Biostatistical Analysis*. 4th edn. Prentice-Hall, Inc., New Jersey, USA.

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