
Occurrence and diversity of thermophilous soil microfungi in forest and cave ecosystems of Taiwan

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The diversity of thermophilous microfungi of soil samples from lowland rainforest and limestone caves in southern Taiwan have been studied. Soil samples were collected from the forest and caves around Kenting National Park, Taiwan. Using a soil dilution plating method, soil samples from each location were inoculated onto yeast phosphate soluble starch agar nutrient medium and the plates were kept at 45 C to screen for the diversity and occurrence of thermophilous fungi. Eleven species of microfungi belonging to eight genera were identified. Mitosporic taxa were represented by seven species, while zygomycetes and ascomycetes were each represented by two species. The number of thermotolerant and thermophilic fungal species were higher in the forest soil than in the entrance, twilight and dark zone areas of the limestone caves. *Aspergillus flavipes* and *Phialophora* sp. were only found in forest soils while *Absidia corymbifera*, *Talaromyces duponti*, *Humicola lanuginosa*, *Mucor pusillus* and *Myriococcum albomyces* were isolated from forest soil samples, as well as at the entrances of caves. Simpson's diversity indexes of fungi were low in twilight and dark zones of the caves and species such as *Aspergillus niger*, *A. tamarii*, *A. wentii*, and *Byssochlamys* sp. were restricted to these areas.

Key words: biodiversity, cave, microfungi, rainforest, Taiwan.

Introduction

Tropical microfungi are remarkable for their antiquity and ubiquitous distribution and they contribute a major role not only in the tropical forest ecosystem functioning but also in the maintenance of biological diversity (Hyde, 1997). Unfortunately, they are one of the poorly studied organisms in the world (Hawksworth, 1991) and most studies are pioneer work restricted to the identification of species, locally or regionally. A variety of mesophilous and thermophilous microfungal species are known to occur in forest soils of tropical environments (Sandhu and Singh, 1981; Agarwal and Chauhan, 1988; Attili and Tauk-Tornisielo, 1994; Pfenning, 1997; Koilraj *et al.*, 1999). In Taiwan, microfungal research is mostly restricted to the identification of pathogens on vegetable crops (Wang *et al.*, 1995; Sivanesan *et al.*, 1998) and very little is known about the microfungal diversity in rainforest ecosystems.

This report for the first time presents data on the diversity of thermophilous microfungi in soil samples collected from different microhabitats of the rainforest and ecological zones of four limestone caves at Kenting National Park, southern Taiwan.

Material and methods

Study site and location of caves

The study was carried at Kenting National Park (21° 91' N; 120° 80' E) in Taiwan. The park was established in January 1984 to give exclusive protection to 17,731 ha of terrestrial lowland rainforest and 14,900 ha of adjacent ocean (Hsu, 1997). The park is located on the southernmost tip of Taiwan and is bordered on three sides by water, the Pacific Ocean to the east, the Bashi Channel to the south, and the Taiwan Strait to the west (Fig. 1). The climate at Kenting is tropical with dry winters and humid summers. The mean monthly temperatures range from 29.8 C (July) to 21.5 C (January), without a cold winter (Hsu, 1997). Precipitation is usually concentrated from May to September and the dry season lasts for about six months with monthly precipitation less than 40 mm.

Cave 1 is situated in the tourism zone. Artificial sodium lamps light up the area and large numbers of tourists visit on a regular basis (Fig. 1). Cave 1 extends to 125.5 m in length (mouth facing 175° southeast) while the other caves (2, 3 and 4) are well hidden by the lowland rainforest vegetation thus escaped the attention of park staff as well as visitors. Cave 2 extends to 68 m (facing 120° southeast), while Cave 3 faces the southwest (190°), and Cave 4 faces southwest (250°). The former extends to 83.5 m and the latter to 45 m from the entrance.

Soil samples collection and analysis

Soil samples (10 g) were collected during January 2000 randomly at five different sites from each zone (forest, entrance, twilight and dark zones of four caves) in sterile containers. All five random samples of each zone were put together to make a single sample for each zone and for each cave. A total of 16 samples (four each from forest, entrance zone, twilight zone and dark zone) were prepared to investigate the diversity of the thermophilous fungi. The ecological analysis of number of propagules and percentage occurrence of each species of fungi in each sample were calculated according to Michael (1984) and Frankland (1990). Spearman rank correlation coefficient (SAS, 1989) was used to test the correlation of the number of propagules of thermophilous fungi from different zones and from forest.

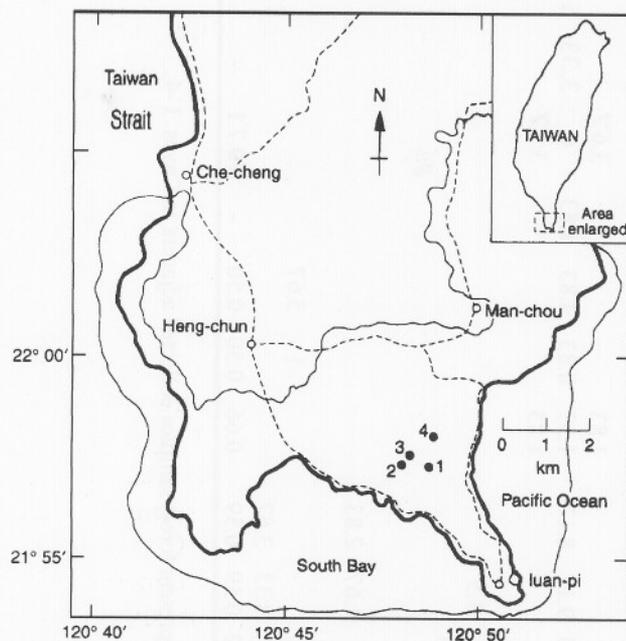


Fig. 1. Map of Kenting National Park and location of Caves 1-4. Open circles represent towns, broken lines represent roads, the thick solid line shows the land boundary and the thin solid line represents the boundary of the park including the marine area.

Results

A total of 11 species of thermophilous fungi belonging to eight genera were isolated from 96 dilution plates made with soil samples collected from forest and entrance, twilight and dark zones of four caves at Kenting. Seven species were mitosporic taxa, and two species each were zygomycetes and ascomycetes (Table 1). The genus *Aspergillus* comprised four species, while the other seven genera were represented by a single species (Table 1). Seven out of the 11 species (*Absidia corymbifera*, *Aspergillus flavipes*, *A. niger*, *A. tamaritii*, *A. wentii*, *Byssochlamys* sp. and *Phialophora* sp.) were thermotolerants. The remaining four (*Humicola lanuginosa*, *Mucor pusillus*, *Myriococcum albomyces* and *Talaromyces duponti*) were thermophilic.

The occurrence and the number of thermophilous fungi were highest in the forest soil samples, as compared to those of the cave samples (Table 1; Fig. 2). Nearly 60-70% of thermophilous fungi were found in the forest soils (average $63.1 \pm \text{SD } 5.2\%$, F1 to F4, 59%, 69.9%, 59% and 64.3% respectively). Moreover, the average relative abundance of thermophilous fungi at the twilight zone ($6.5 \pm 4.8\%$) was smallest, as compared to those from the entrance ($20.4 \pm 1.9\%$) and dark zone ($10.1 \pm 4.4\%$) samples of caves. The

Table 1. The occurrence of average number of thermophilous fungal propagules/gram ($\times 10^3$) and Simpson's Diversity Index in dry soil samples collected from forest and different ecological zones of caves at Kenting National Park.

| Fungi | Forest | | | | Entrance zone | | | | Twilight zone | | | | Dark zone | | | |
|----------------------------------|--------|------|------|------|---------------|------|------|------|---------------|------|------|------|-----------|------|------|-----|
| | F1 | F2 | F3 | F4 | C1 | C2 | C3 | C4 | C1 | C2 | C3 | C4 | C1 | C2 | C3 | C4 |
| Mitosporic taxa | | | | | | | | | | | | | | | | |
| <i>Aspergillus flavipes</i> (tt) | 8.33 | | | | | | | | | | | | | | | |
| <i>A. niger</i> (tt) | 7.17 | 6.5 | 13.3 | 9.67 | 3.83 | | | | 1.83 | | | | 3.67 | | | |
| <i>A. tamarii</i> (tt) | 7.50 | 5.5 | 12.5 | 10.8 | 3 | 3.83 | 9.17 | 8.17 | 1.33 | 0.83 | 4.83 | 1.83 | 6 | 3.33 | 6.17 | 6.3 |
| <i>A. wentii</i> (tt) | 3.83 | | | | 3.83 | | | | 1.83 | | | | 3.17 | | | |
| <i>Phialophora</i> sp. (tt) | | 8.33 | 8 | 8 | | | | | | | | | | | | |
| <i>Talaromyces duponti</i> (t) | 2.33 | | 2.5 | | 0.83 | | 1.5 | | | | | | | | | |
| <i>Humicola lanuginosa</i> (t) | 7.67 | 6.83 | | | | | 3.33 | | | | | | | | | |
| Zygomycetes | | | | | | | | | | | | | | | | |
| <i>Absidia corymbifera</i> (tt) | 2.00 | 1.33 | | | 1 | | | | | | | | | | | |
| <i>Mucor pusillus</i> (t) | | | 3.33 | 4.33 | | | 1.67 | 2.83 | | | | | | | | |
| Ascomycetes | | | | | | | | | | | | | | | | |
| <i>Byssochlamys</i> sp. (tt) | | 5.17 | | | | 2.17 | | | | 1 | 5.67 | | | | | |
| <i>Myricoccum albomyces</i> (t) | 8.17 | 3.5 | 7 | 8.33 | 2.17 | 1.5 | 3.33 | 3.67 | | | | | | | | |
| Simpson's Diversity | 0.85 | 0.83 | 0.79 | 0.79 | 0.79 | 0.72 | 0.59 | 0.59 | 0.66 | 0.50 | 0.50 | - | 0.71 | - | - | - |

tt = thermotolerant; t = thermophilic; C1-4 represent Caves 1-4; F1-F4 represent forest samples collected adjacent to Caves 1-4.

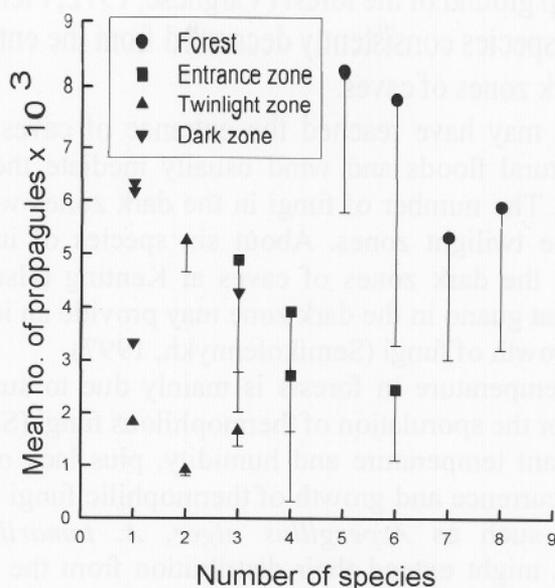


Fig. 2. The occurrence and diversity of thermophilous microfungi (per gram of dry soil) in different ecological zones of four caves and forest sites at Kenting National Park, Taiwan.

number of propagules of thermotolerant fungi in the forest was significantly correlated with those in the entrance and dark zones (Spearman correlation coefficient $r = 0.43, 0.45$, respectively, $n = 28$, $p < 0.05$), but not correlated with that of the twilight zones ($p > 0.12$). Only *Byssochlamys* sp. occurred in the twilight zone of Cave 3, but did not occur in the nearby forest and entrance or dark zones of Cave 3. The Simpson's Diversity of thermophilous fungi was highest in soils from forest and decreased from the entrance zone to the dark zone except in Cave 1 (Tables 1). One species, *Aspergillus tamaris* was invariably isolated from all areas. Thermophilic fungi did not occur at the twilight and dark zones, but four species of thermotolerant fungi were found both in the forest and cave samples. In addition, the relative abundance of *A. niger*, *A. tamaris* and *A. wentii* was higher in the dark zones as compared to that of the twilight zones.

Discussion

The genus *Aspergillus* was found to be the most abundant in forest and cave soil samples. This may be due to its ubiquitous distribution in nature (Maheshwari, 1996). The number of fungal species in the forest sample was higher than the cave samples and the high diversity could have been due to the enriched decaying organic matter. Similarly, the topsoil layers of the rainforest was reported to have higher number of fungal species as compared to the lower

horizon in the deep ground of the forest (Varghese, 1972; Pfenning, 1997). The number of fungal species consistently decreased from the entrance to twilight, and twilight to dark zones of caves.

Fungal spores may have reached the entrance of caves via water or air currents since natural floods and wind usually mediate the entry of fungal spores into caves. The number of fungi in the dark zones was comparatively higher than in the twilight zones. About six species of insectivorous bats regularly roost in the dark zones of caves at Kenting (Hsu, 1997) and the accumulation of bat guano in the dark zone may provide an ideal environment to stimulate the growth of fungi (Semikolennykh, 1997).

Increases in temperature in forests is mainly due to sunlight, solar heat being necessary for the sporulation of thermophilous fungi (Sandhu and Singh, 1981). The constant temperature and humidity, plus lack of light, however, may affect the occurrence and growth of thermophilic fungi in the dark zones of caves. Fungi such as *Aspergillus niger*, *A. tamarii*, *A. wentii* and *Byssochlamys* sp. might extend their distribution from the forest soil to the twilight or dark zones of caves. Thermophilic fungi however, were not isolated from the twilight and dark zones of the caves and their absence is likely to be due to the lack of light combined with the less variable temperatures. Thus it appears that sunlight and heat are essential for the dispersal and sporulation of thermophilic fungi as suggested by Bell-Pederson *et al.* (1996).

When Sandhu and Singh (1981) isolated thermophilous fungi from low, median and high altitude forest soils, they found out that the maximum number of fungi were from the low hill zone where the annual temperature fluctuation was favorable for the growth of fungi. In this study, thermophilic fungi were isolated only from entrance of caves (i.e. that is not inside caves), where temperature, humidity and light fluctuate daily. These environmental parameters were nearly constant in twilight and dark cave areas and could have discouraged thermophilous fungal growth.

Tropical microfungi, especially thermophilous fungi, represent an unexplored universe of biodiversity and these fungal opportunists produce a vast range of enzymes that can degrade many kinds of organic and inorganic substrates in delicate rainforest and limestone cave environments (Hyde, 1997). The widespread nature of microfungi represents a challenge to those who desire to understand their biodiversity and their specific role in the maintenance of forest ecosystems. This largely invisible world is even more mysterious and unknown in tropical regions than in temperate regions.

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