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The Mycoflora of Hot Spring Soil in Northern Taiwan

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ABSTRACT: An investigation of the mycoflora in northern Taiwan from August 1999 to June 2000, particularly of thermophilic and thermotolerant fungi inhabiting sulfurous hot spring soils, resulted in identification 12 taxa: *Aspergillus fumigatus* var. *fumigatus* (66.85 %), *A. fumigatus* var. 1 with green colony (7.86 %), *A. fumigatus* var. 2 with brown colony (4.81 %), *A. niger* (1.14 %), unidentified *Asperigillus* sp. (0.045 %), *Chrysosporium* sp. (0.18 %), *Papulaspora thermophila* (2.72 %), *Scytalidium thermophilum* (0.045 %), *Sporotrichum* sp. (0.045 %), *Mycelia sterilia* sp.1 with white colony (6.63 %), *Mycelia sterilia* sp.2 with yellow colony (5.27 %) and *Mycelia sterilia* sp. 3 with gray colony (4.405 %). A total of 2202 colonies were isolated from three sampling sites: site 1 (hot springhead), site 2 (2 m from site 1) and site 3 (4 m from site 1). Fungal colonies isolated as well as species percentage at three sites were as follows: 32.92 % in 9 taxa from site 1, 37.87 % in 11 taxa from site 2, and 29.21 % in 8 taxa from site 3. The dominant species was *Aspergillus fumigatus* var. *fumigatus*, which was isolated year around from three sampling sites. *A. fumigatus* var. 1 appeared from February to June 2000. *A. fumigatus* var. 2 was isolated only in August and October 1999. Within the sampling range of hot spring niches, there was evidence of the presence of ecotypes in the *A. fumigatus* complex. *Chrysosporium* sp. and *Sporotrichum* sp. were isolated only from the soils without hot water treatment, but *Aspergillus* sp. and *Scytalidium thermophilum* were isolated only from the soils pre-treated with hot water for 30 min. at 60℃. The significance level (P value) of fungal communities between hot water treatment and no treatment was 0.866, indicating that no significant difference between both treatments.

KEY WORDS: Mycoflora, Thermophilic fungi, Thermotolerant fungi, Sulfurous hot spring soils.

INTRODUCTION

Yangmingshan National Park is located in the northern Taiwan and has volcanic geology, abundant minerals, and northeast monsoon for about half the year. The average monthly temperature ranges from 10.2℃ to 25.3℃, with relative humidity between 79% and 94%; precipitation ranges from 33.1 mm to 1181.9 mm, with wind speed from 0.7 to 2.9 m/sec. Volcanic and post-volcanic landscapes support vegetative zones of high grassland, low grassland, warm temperate forest, evergreen broadleaf forest, subtropical rain forest, and aquatic plant communities containing diverse plant and animal life**.** As a result of post-volcanism, active geothermal systems, extrusive sulfur, and acidic hot springs occur in the areas around Hsiaoyukeng, Mataso, and Tayukeng (Liang, 1990; Lin, 2000; Tsai & Sha, 2001).

Hsiaoyukeng is one of Yangmingshan National Park's major geologic scenic areas. Strongly acidic hot springs and sulfur gas rising through the Hsiaoyukeng fault have eroded the extrusive rock and caused it to turn crumble and collapse, producing strongly acidic soils and significant accumulation of saltsis in the area. In such extreme environment, no plant life survives. (Chen, 1989; Chou & Li, 1991; Liang, 1990). Besides strongly acidic and saltsis soils, soil temperature at the Hsiaoyukeng sulfur hot spring area is 50℃ or higher.

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Soil is a natural medium for fungal growth and reproduction (Weng *et al*., 1991), therefore, the number of propagules and the diversity of fungal species in soils reflect variation in physical and chemical conditions of soils (Dix and Webster, 1995; Fries, 1973). Fungi are heterotrophic eukaryotes which exhibit great diversity in morphology and distribution. Some species can survive in various adverse environments such as hot springs (Brock, 1978) or geothermal soils (Redman *et al*., 1999) etc. Normally, the growth temperatures for the majority of fungi is between 25℃ and 30℃, above 40℃ growth is poor or mortality occurs. Some species can grow at temperatures above 50℃: those unable to grow below 20℃ are called thermophiles, while those able to grow below 20℃ are called thermotolerant (Cooney & Emerson, 1964).

The first of the known thermophilic fungi, *Dactylaria gallopava* (W. B. Cooke) Bhatt *et* Kendrick, was isolated from hot springs effluents by Tansey and Brock (1973). Until 2000, five species of thermophilic fungi have been described from hot springs water (Chen *et al*., 2000). However, the soil fungal flora in sulfurous hot spring areas is not well known. In Taiwan, only Chen *et al*. (2000) have investigated the fungal flora of Wu-Rai hot springs water. The present study was undertaken to investigate the occurrence of thermophilic and thermotolerant soil mycoflora and soil factors which may affect the distribution and types of soil fungi.

MATERIALS AND METHODS

Sampling area and sample collection

The sampling area was located at the Hsiaoyukeng sulfur hot spring area of Yangmingshan National Park, Taipei, Taiwan. Three plots were set up by line method (Rossman *et al*., 1998): site 1 at the hot springhead, site 2 two meters from site 1 and site 3 four meters from site 1.

Soil samples were taken in each point from depths of 0 cm to 15 cm by using a soil liner sampler (Eijkelkamp, Agrisearch Equipment) from August 1999 to June 2000 at two-month intervals. The soil samples were air-dried for sulfate and organic content analysis.

Fungal isolation and identification

Soil samples from each site on each date were divided into control (untreated, direct plating) and treatment subsamples (treated with hot water). Two grams of treatment soil sample were poured into a 10 mL sterile test tube with 4 mL distilled water and shaken at 60℃ in a hot water bath for 30 min (Chen and Chen, 1988). Following treatment, the samples were transferred to 9 cm petri dishes containing five different media and incubated for 3-10 days at 45℃. The media were: (1) corn meal agar (CMA-corn meal 20 g, pepton 20 g, dextrose 20 g, agar 15 g, 1000 mL distilled H_2O); (2) Czapek agar (CZA-sucrose 30 g, $NaNO_3$ 3 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, KCl 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, agar 15 g, 1000 mL distilled H_2O) with 4 g yeast extract; (3) rose bengal medium (RBM-dextrose 10 g, yeast extract 0.5 g, pepton 0.5 g, K_2HPO_4 0.5 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, rose bengal 0.05 g, streptomycin 0.03 g, agar 17 g, 1000 mL distilled H_2O); (4) yeast extract soluble starch agar (YpSs-yeast extract 4 g, soluble starch 15 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar 15 g, 1000 mL distilled H_2O); and (5) sulfide indole motility medium (SIM medium-pancreatic digest of casein 20 g, peptic digest of animal tissue 6 g, sodium thiosulfate 0.3 g, ferric ammonium citrate 0.2 g, agar 3.5 g, 1000 mL distilled H_2O) containing chloramphenicol 100 mg/L. Colonies on each plate were counted and pure cultured for identification.

Soils analysis

Temperature and moisture The temperature and moisture were tested directly at sampling sites by using a Moisture-Temp meter (Aquater TEMP-200: Aquater Instruments, Inc., CA).

pH The pH was determined directly at sampling sites using a pH meter (Sentron 1001: Sentron Europe B.V.).

Organic content The 0.5 g air-dried soil sample was mixed with 10 mL 1N $K_2Cr_2O_7$, and 20 mL of concentrated H_2SO_4 was added with constant stirring. The sample was allowed to stand for 30 min, then 200 mL of distilled water and 10 ml of 85% phosphoric acid were added. When the solvent cooled, 2-3 drops of diphenylamine reagent was added and mixed with the solution, then 0.5N FeSO4 was pipetted into the solution with constant stirring until the solution turned green. Organic content was calculated using the following formula (Kuo, 1976): Organic content (%) = $10 \times (1-S/B) \times 1.0 \times 12/4000 \times 1.724/0.77 \times 100$/soil weight (g) (S: titrant sample volume of FeSO4; B: titrant blank volume of $FeSO_4$; 10 : 1 (v/v) = volume of $K_2Cr_2O_7$: concentration of $K_2Cr_2O_7$; 1.724: Ven Bemmelen factor; 0.77: recovering rate of this procedure).

Sulfate test Soil samples were extracted with $CaHPO_4$ (500mL/LP) solution, and the extract was diluted with 200-400 parts of distilled water. SulfaVer 4 sulfate reagent (HACH method 10084, Hach Company, U.S.A.) was added, shaken, and absorbance was measured at 680 nm. Actual absorbance and absorbance unit were converted to g/m^2.

Data analysis The total number of species at each sampling site and their percentages were recorded. The total fungal colonies at each site were compared on the basis of Similarity Index and the species colonies resulting from two isolating methods were analyzed by using one-way ANOVA. The relationship of soil factors and fungal species was determined by correlation analysis.

RESULTS

Species composition

Isolated genera and species of fungi from hot spring soils were listed in Tables 1 and 2. Twelve taxa were isolated: *Aspergilus fumigatus* var. *fumigatus* (66.85%), *A. fumigatus* var. 1 with green colony (7.86%), *A. fumigatus* var. 2 with brown colony (4.81%), *A. niger* (1.14%), unidentified *Asperigilus* sp. (0.045%), *Chrysosporium* sp. (0.18%), *Papulaspora thermophila* (2.72%), *Scytalidium thermophilum* (0.045%), *Sporotrichum* sp. (0.045%), *Mycelia sterilia* sp. 1 with white colony (6.63%), *Mycelia sterilia* sp. 2 with yellow colony (5.27%) and *Mycelia sterilia* sp. 3 with gray colony (4.405%). A total of 2202 colonies were isolated from the three sampling sites. Fungal species number of colony and percentage (%) at the three sites were as follows: 32.92% in nine taxa from site 1, 37.87% in eleven taxa from site 2, and 29.21% in eight taxa from site 3 (Table 2).

Table 1. Genus number of colony and percentage (%) and seasonal variation in Hsiaoyukeng sulfur hot spring area during August 1999-June 2000.

Genera	1999			2000			No. of colony	Percentage (%)
	Aug	Oct	Dec	Feb	Apr	June		
Aspergillus	234	205	353	374	288	323	1777	80.70
Chrysosporium	0	4	0	0	0	0	4	0.18
Papulaspora	1	9	20	30	0	0	60	2.72
Sporotrichum	0	0	0	0	1	0	1	0.05
Scytalidium	0	0	0	1	0	0	1	0.05
Mycelia	45	22	32	120	56	84	359	16.30
Total	280	240	405	525	345	407	2202	

Table 2. Species number of colony and percentage (%) in soils of different sampling sites in Hsiaoyukeng sulfur hot spring area.

Species	Sampling areas			No. of colony	Percentage (%)
	Site 1	Site 2	Site 3		
Aspergillus fumigatus var. *fumigatus*	342*	743	387	1472	66.85
A. fumigatus var. 1 (green colony)	45	48	80	173	7.86
A. fumigatus var. 2 (brown colony)	82	2	22	106	4.81
A. niger	15	6	4	25	1.14
A. sp.	0	1	0	1	0.05
Chrysosporium sp.	4	0	0	4	0.18
Papulaspora thermophila	32	7	21	60	2.72
Scytalidiumn thermophilum	0	1	0	1	0.045
Sporotrichum sp.	0	1	0	1	0.045
Mycelia sterilia sp. 1 (white colony)	90	20	36	146	6.63
M. sterilia sp. 2 (yellow colony)	36	3	77	116	5.27
M. sterilia sp. 3 (gray colony)	79	2	16	97	4.405
Total	725	834	643	2202	
Percentage (%)	32.92	37.87	29.21		

*: Species number of colony

Seasonal variation

Occurrence of different fungal taxa varied with season. The dominant species *Aspergilus fumigatus* var. *fumigatus* was isolated year-round from the three sampling sites. *A. fumigatus* var. 1 (green) appeared from February to June (2000). *A. fumigatus* var. 2 (brown) was isolated only in August and October (Table 1 and Figure 1).

Sampling sites

Within the sampling range of the hot spring niche studied, there is an evidence of presence of the existence ecotypes in the *A. fumigatus* complex, including *Aspergillus fumigatus* var. *fumigatus*, *A. fumigatus* var. 1 with green colony, *A. fumigatus* var. 2 with brown colony (Table 2). The similarity of fungal species between three sampling sites was high (Table 3).

Table 3. Similarity* of fungal species of different sampling areas in Hsiaoyukeng sulfur hot spring area.

Sampling areas	Site 1	Site 2	Site 3
Site 1		0.8	0.823529
Site 2	0.8		0.84210
Site 3	0.823529	0.84210	

*S=2C/(A+B)
A= number of species in sample A.
B= number of species in sample B.
C= number of species common to both samples
S=0(low)~1(high)

Soil factors

Soil environmental data to a depth of 15 cm is shown in Table 4. The pH values of all samples were in the range 1.7~3.2. The average temperature at 15 cm depth was 57℃± 4.47 ℃ at site 1, 50℃± 16.149℃ at site 2 and 35℃± 14.255℃ at site 3. Sulfate contents at sites 1, 2, and 3 were 292.096 g/m^2, 244.608 g/m^2, and 169.214 g/m^2. Organic contents were 3.15%, 3.50% and 2.03%. Relative humidity were 58.8 ± 7.1386%, 61.2 ± 12.528%, 86 ± 6.066%.

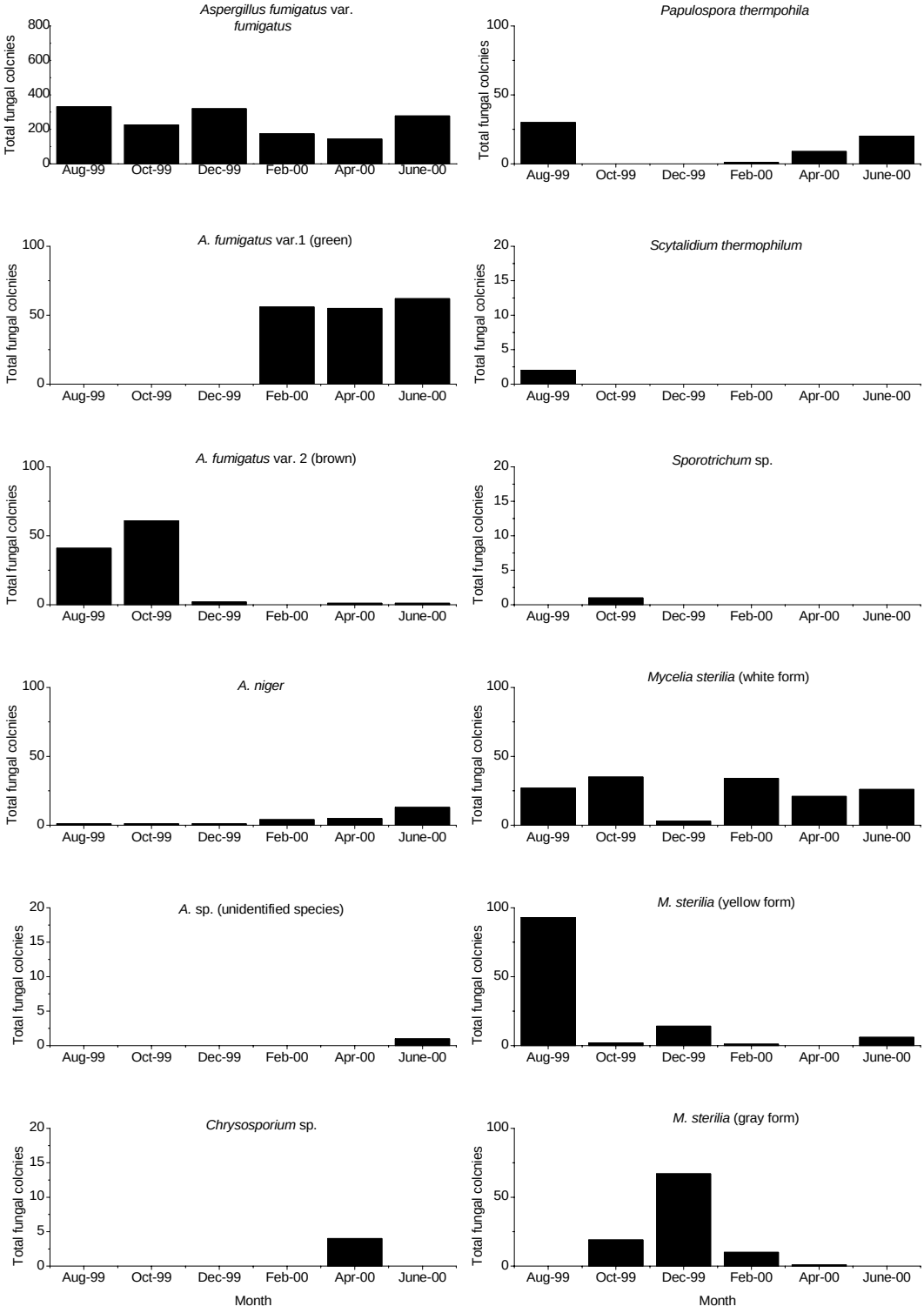

Fig. 1. Relationships between the number of different fungi isolated and seasonal variation.

Table 4. Soil environmental data in different sampling areas in Hsiaoyukeng sulfur hot spring area during August 1999-June 2000 from depths of 0-15 cm.

Soil factors	Sampling areas		
	Site 1	Site 2	Site 3
pH (90 mins)	1.7~2.7 (Ave. 2.07)	1.7~3.2 (Ave. 2.26)	6~2.6 (Ave. 2.07)
Sulfate (g/m^2)	292.096	244.608	169.214
Organic contents (%)	3.15	3.50	2.03
Relative humidity (%)	58.8±7.1386	61.2±12.528	86±6.066
Aveage temperature (℃) (15 cm deep soil site)	57℃±4.47	50℃±16.149	35℃±14.255

Hot water treatment

C*hrysosporium* sp. and *Sporotrichum* sp. were isolated only from the soils without hot water treatment but *Aspergilus* sp. and *Scytalidium thermophilum* were isolated only from the soils pre-treated with hot water bath (Table 5). The significance level (P value) of the fungal community between hot-water treatment and no treatment was 0.866, indicating that no significant difference of both treatments in the present investigation (Table 6).

Table 5. Fungi isolated from two isolating methods (hot water treatment: 60℃, 30 mins. and control: untreated, direct plating).

Genera	Hot water treatment	Control
Aspergillus fumigatus var. *fumigatus*	800	672
A. fumigatus var. 1 (green)	85	88
A. fumigatus var. 2 (brown)	2	104
A. niger	18	7
A. sp.	1	0
Chrysosporium sp.	0	4
Papulaspora thermophila	2	58
Scytalidium thermophilum	1	0
Sporotrichum sp.	0	1
Mycelia sterilia sp. 1 (white colony)	50	95
M. sterilia sp. 2 (yellow colony)	18	98
M. sterilia sp. 3 (gray colony)	37	60

Table 6. Comparison of the results of two isolating methods (hot water treatment: 60℃, 30 mins. and control: untreated, direct plating) by using one way ANOVA.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1247.042	1	1247.042	0.029	0.866
Within Groups	944457.920	22	42929.905		
Total	945704.960	23			

Sig. = P-level ($P < 0.05$)

DISCUSSION

In this study, fungal communities in soil usually showed seasonal variation, these results were similar to the study by Bissett and Parkinson (1979). The correlation between soil factors and species in this investigation, sulfate was the main inhibator factor for *Aspergillus fumigatus* var. *fumigatus* (R = - 0.708), correlated negatively with measure of sulfur contents. The main reason is that the relatively low number of other isolates was not analyzed.

According to Christensen (1989) and Weng *et al*. (1991), soil fungal diversity is associated with the plants of different ecosystems. Hsiaoyukeng sulfurous hot spring area due

to high temperature, high acidity, low nutrients and high sulfate contents, plants were absent or very rare in the extrusive gas aperture. These sampling areas represented an oligotrophic environments (Jennings, 1993). From these results, soil in this area was a poor medium for fungal growth and showed relatively low fungal species diversity than other areas such as Luchiaokengshi broadleaf forest, Hsiaoyukeng arrow bamboo bushes, and silvergrass grassland in Yangmingshan National Park (Chien *et al*., 2000).

Compared with some other ecosystems containing various fungal genera in the Zygomycetes (*Rhizomucor pusillus, Rhizopus rhizopodiformis*), Ascomycetes (*Emericella nidulans, Neosartorya fischeri* var. *spinosa, Thermoascus aurantiacus, Thielavia heterothalica* etc.), and Deuteromycetes (*Humicola hyalothermophila, Malbranchea sulfurea, Myceliophthora thermophilum, Paecilomyces variotii* etc.) (Chien *et al*., 2000), the microbial community in the sulfur hot spring area of Hsiaoyukeng was simple and consisted wholly of Deuteromycetes (most *Aspergillus fumigatus* complex).

This study revealed that some fungi such as *Aspergillus* spp. can grow in very low nutrient flux environments, it may be that such fungi can get nutrients from the atmosphere or from windblown soil and dust (Staley *et al*., 1982). Wainwright (1988) pointed out fungi can use a wide variety of nutrients, including carbon monoxide, or hydrocarbons etc., in which occurred in the soil atmosphere or air, and provided sufficient nutrients for fungal growth under low nutrient coniditions.

The result of hot water treatment revealed no significant difference between fungal community resulted, the reason might be that these isolates themselves are all heat-tolerant fungi.

In the course of the present study, the following four thermophilic fungal species were first isolated at Hsiaoyukeng sulfurous hot spring area: *Chrysosporium* sp.*, Papulaspora thermophila, Scytalidium thermophilium,* and *Sporotrichum* sp. The species *A. niger*, *Chrysosporium* sp., *P. thermophila, S. thermophilum* and *Sporotrichum* sp. were isolated only once. These preliminary results provide information on soil fungal species diversity in hot sulfur areas and correlation of sulfate content and fungal species.

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北臺灣溫泉區土壤之真菌相

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摘　　要

從 1999 年 8 月到 2000 年 7 月，本實驗調查北臺灣硫磺熱溫泉區土壤的高溫真菌相，結果共分離到十二種分類群：*Aspergillus fumigatus* var. *fumigatus* (66.85 %), *A. fumigatus* var. 1 (綠色菌落) (7.86 %), *A. fumigatus* var. 2 (褐色菌落) (4.81 %), *A. niger* (1.14 %), unidentified *Asperigillus* sp. (0.045 %), *Chrysosporium* sp. (0.18 %), *Papulaspora thermophila* (2.72 %), *Scytalidium thermophilum* (0.045%), *Sporotrichum* sp. (0.045%), *Mycelia sterilia* sp. 1 (白色菌落) (6.63%), *Mycelia sterilia* sp. 2 (黃色菌落) (5.27%) 和 *Mycelia sterilia* sp. 3 (灰色菌落) (4.405 %). 由三個採樣位置的土壤：位置 1 (熱溫泉頭)，位置 2 (離位置 1，二公尺處)，位置 3 (離位置 1，四公尺處)，共分離出 2202 個菌落。而菌種之百分比分別為：位置 1 有九種分類群，佔 32.92 %；位置 2 有十一種分類群，佔 37.87 %；位置 3 有八種分類群，佔 29.21%。優勢種 *Aspergillus fumigatus* var. *fumigatus* 於三個採樣點全年均可分離到。*A. fumigatus* var. 1 出現於 2000 年二月和七月。*A. fumigatus* var. 2 出現於 1999 年八月和十月。在採樣區熱溫泉生態區，發現 *A. fumigatus* complex 具有生態型。*Chrysosporium* sp. and *Sporotrichum* sp. 僅出現在未經熱水處理的土壤，而 *Aspergillus* sp. and *Scytalidium thermophilum* 出現在經熱水處理過的土壤 (30 min, 60℃)。未經熱水處理的土壤與事先經熱水處理過的土壤，真菌群落之 P 值為 0.866，顯示兩種處理後結果差異性不大。

關鍵詞：真菌相、嗜熱性真菌、耐熱性真菌、硫磺熱溫泉區土壤。

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