The Journal of Microbiology (2010) Vol. 48, No. 2, pp. 146-152
DOI 10.1007/s12275-010-9157-2

Diversity of Thermophilic Fungi in Tengchong Rehai National Park Revealed by ITS Nucleotide Sequence Analyses

Wen-Zheng Pan†, Xiao-Wei Huang†, Kang-Bi Wei, Chun-Mei Zhang, Dong-Mei Yang, Jun-Mei Ding, and Ke-Qin Zhang*

Laboratory for Conservation and Utilization of Bio-Resources, and Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, Yunnan, 650091, P. R. China

(Received May 13, 2009 / Accepted October 20, 2009)

The geothermal sites near neutral and alkalescent thermal springs in Tengchong Rehai National Park were examined through cultivation-dependent approach to determine the diversity of thermophilic fungi in these environments. Here, we collected soils samples in this area, plated on agar media conducive for fungal growth, obtained pure cultures, and then employed the method of internal transcribed spacer (ITS) sequencing combined with morphological analysis for identification of thermophilic fungi to the species level. In total, 102 strains were isolated and identified as *Rhizomucor miehei*, *Chaetomium* sp., *Talaromyces thermophilus*, *Talaromyces byssochlamydoides*, *Thermoascus aurantiacus* Miehe var. *levisporus*, *Thermomyces lanuginosus*, *Scytalidium thermophilum*, *Malbranchea flava*, *Myceliophthora* sp. 1, *Myceliophthora* sp. 2, *Myceliophthora* sp. 3, and *Coprinopsis* sp. Two species, *T. lanuginosus* and *S. thermophilum* were the dominant species, representing 34.78% and 28.26% of the sample, respectively. Our results indicated a greater diversity of thermophilic fungi in neutral and alkaline geothermal sites than acidic sites around hot springs reported in previous studies. Most of our strains thrived at alkaline growth conditions.

***Keywords*:** thermophilic fungi, geothermal soils, morphological study, molecular study, physiological study, diversity

Specific genetic and physiological adaptations allow microorganisms to exist in a variety of environments that experience extremes of temperature, pH, chemicals, and pressure (Cooney and Emerson, 1964; Aguilar, 1996; Mouchacca, 1997; Stetter, 1999). Thermophilic organisms may belong to any of the three domains of life. However, most thermophilic species described so far are members of the Archaeal or Eubacterial domains (Barns *et al*., 1996; Hugenholtz *et al*., 1998). Among the eukaryotic organisms, only a few species of fungi have been found to thrive at temperatures between 45 and 55°C. Based on their minimum and maximum temperatures of growth, these fungi are grouped into thermophilic and thermotolerant forms (Cooney and Emerson, 1964): the thermophilic group has a minimum growth temperature of above 20°C and grow well at even higher than 50°C; while the thermotolerant group has a temperature range of growth from below 20°C to 55°C.

Thermophilic fungi were first reported in 1899 by P. Tsiklinsky, and many of them were isolated from guayule undergoing retting during rubber production, a process similar to composting (Cooney and Emerson, 1964). Since then, over 28 species of thermophilic fungi have been well described (Mouchacca, 1997; Maheshwari *et al*., 2000), and their sampling sites include manure composting, industrial coal mine soils, beach sands, nuclear reactor effluents, Dead Sea valley soils, and desert soils of Saudi Arabia (Redman *et al*., 1999). In addition, fungi from natural geothermal soils have recently been investigated, including fungal communities in geothermal soils around sulfurous hot springs (Redman *et al*., 1999; Chen *et al*., 2003).

Classical taxonomy based on colony morphology, hyphal structure, and spore arrangement have placed the thermophilic fungi into the following classes, i.e. zygomycete, ascomycete, and hyphomycete (Mouchacca, 1997). However, morphological differences within strains grown on different media types and cultural conditions are often observed. Moreover, the practice of interchangeably using different names (anamorph and teleomorph stages) for same fungus has also resulted in confusion in identifying these fungi (Maheshwari *et al*., 2000). Therefore, the molecular analysis based on DNA sequences, recognized as the most reliable methods to reveal genetic relationships between the strains, could avoid such problems and unambiguously place the isolates at any taxonomic rank (Bruns *et al*., 1991). Different molecular targets have been used for species identification, including conserved ribosomal RNA genes and the more variable internal transcribed spacer (ITS) regions (Schwarz *et al*., 2006; Sharma *et al*., 2008). In this study, ITS sequencing combined with morphologic observation was used for identification of thermophilic fungi to the species level.

Tengchong Rehai National Park, one of China's best known volcanic geothermal areas, is located in southwestern China. It is on the India plate collision with the European plate convergence zone and is an integral part of the Mediterranean-Himalayan geothermal belt. As a result of post-volcanism and an active geothermal system, there are many neutral and alkalescent thermal springs in this area, where quite a few

† These authors contributed equally to this work. * For correspondence.
E-mail: kqzhang111@yahoo.com.cn; Tel & Fax: +86-871-503-4878

thermophilic bacteria have been isolated and described (Bao *et al*., 2002; Chen *et al*., 2002; Lin *et al*., 2002, 2005). However, the thermophilic fungal community has not been studied in this area. Here we investigated the thermophilic fungi inhabiting in geothermal soils around neutral and alkalescent thermal springs in this region. The objectives of this study were (i) to determine the diversity of cultivable thermophilic fungal species in Tengchong Rehai National Park geothermal soils, (ii) to determine the predominant genus in this area, and (iii) to examine the thermophilic fungal species composition and their physiological characteristics under selected stressful conditions similar to the neutral and alkalescent thermal springs.

Material and Methods

Sampling area and sample collection

A total of 46 soil samples were collected from three geothermal sites near Ha Ma Zui Spring (site 1), Yan Jing Quan Spring (site 2), and Gu Ming Quan Spring (site 3) in Tengchong Rehai National Park. These sites exhibited heterogeneity with regard to temperature and pH. The temperature at the sampling sites varied from 47°C to 71°C, and the pH from 7.5 to 9.0 (site 1: 47-58°C, pH 7.5-7.9; site 2: 62-71°C, pH 8.4-9.0; site 3: 53-66°C, pH 8.0-8.6). Soil samples were taken in each site from depths of 0 cm to 15 cm using a soil liner sampler, and then the samples were aseptically transferred to sterilized plastic bags, mixed thoroughly, stored at 4°C, and processed within a few hours.

Fungal isolation and maintenance

Four culture media were used to obtain fungi from the geothermal soils: (1) corn meal agar (CMA-corn meal 20 g, pepton 20 g, dextrose 20 g, agar 15 g, 1000 ml DW); (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 DW) with 4 g yeast extract; (3) 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 DW); and (4) potato dextrose agar (PDA: potato 200 g, dextrose 20 g, agar 15 g, 1000 ml DW). All media contained 50 mg of streptomycin per liter and 50 mg of ampicillin per liter to inhibit bacterial growth.

Two isolation methods were used (Lyons *et al*., 2000). The first was based on a series of dilutions: soil sample (1 g) was blended with sterile distilled water (SDW, 100 ml) for 2 min (Waring Commercial blender), followed by five serial dilutions with sterilized water. All plates were incubated in the dark at 50°C for 4 days. During this period, plates were checked daily for the presence of thermophilic fungi. The other method was to directly plate soil samples onto 9 cm Petri dishes containing the four different media. The plates were incubated as before and checked for the presence of thermophilic fungi after 4 days.

All of the fungal species were observed grew on PDA. So each morphologically unique fungal colony during isolation was subcultured onto PDA plates, and then transferred to PDA slants for storage at 4°C.

DNA extraction

Complete genomic DNA was isolated as described by Voigt *et al*. (1999) with some modifications. The strains were grown at 50°C in PDA contained in Erlenmeyer flasks and shaken at 200 rpm. Approximately 100 mg of mycelium were collected by filtration through nytal mesh (42 μm pore size), washed with distilled water, blotted with paper towels, frozen with liquid nitrogen and ground to a fine powder with a mortar and pestle. The powder was incubated for 1 h at 65°C in 1 ml CTAB extraction buffer (100 mM Tris-HCl; pH 8.4, 1.4 M NaCl, 25 mM EDTA, 2% CTAB). After that, the lysate was extracted with a phenol-chloroform-isoamyl alcohol solution (25:24:1) and DNA was then recovered by iso-propanol precipitation. The pellet was washed with 70% (v/v) ethanol, dried under vacuum and resuspended in 100 μl TE buffer (10 mM Tris-HCl; pH 8.4, 1 mM EDTA). Genomic DNA was stored at -20°C.

PCR amplifications and sequence analysis

The ITS rRNA and 5.8S rRNA genes were amplified as described by Gené *et al*. (1996) with a Perkin-Elmer 2400 thermal cycler (Perkin-Elmer Cetus Co., USA). The ITS5 and ITS4 primers (White *et al*., 1990) were used. The amplification program consisted of pre-denaturation at 94°C, 5 min; 30 cycles at 95°C, 30 sec; 50°C, 1 min and 72°C, 1 min; and a final extension at 72°C for 7 min. The final products were analyzed by electrophoresis on 1% agarose MP (Boehringer-Mannheim) and cleaned following the GeneClean II protocol (BIO 101). The molecular weights of amplified DNA were estimated by comparison with 100 bp DNA ladder (Gibco-BRL) standard lane.

The sequencing of PCR products was performed on a 3730 DNA sequencer (Applied Biosystems). The ITS sequences of different fungi were aligned to each other as well as the other sequences retrieved from NCBI databases, using multiple sequence alignment software (CLUSTAL X). Dendrograms were generated using the neighbour joining (NJ) method and the boot strapping was carried using 1,000 replications. The ITS sequences from the representative strains in different species were deposited in GenBank under accession numbers FJ548824 to FJ548839 (Table 2).

Morphological study

Taxonomic identification by morphology of the fungal isolates was mainly based on the criteria described in following sources: Cooney and Emerson, (1964), Apinis (1967), Millner *et al*. (1977), Oorschot (1977), Schipper (1978), Ellis (1981, 1982), Upadhyay *et al*. (1984), Straatsma and Samson (1993), Chen and Chen (1996), Guarro *et al*. (1996), Mouchacca (1997).

The range and optimal temperature for fungal growth

The temperature range suitable for the growth of each fungus was determined by observing whether they could grow or not after incubation of PDA plates for 24 h to 96 h at 15, 20, 25, 30, 35, 40, 50, 55, 60, and 65°C, respectively. The species that grew at or above 50°C but not below 20°C were considered thermophilic fungi. However, the species that grew at 50°C and also at or below 20°C were considered thermotolerant, and the mesophilic species were defined as those capable of growing at temperatures between 20 and 40°C and could not tolerate 55°C. The optimum temperatures of thermophilic isolates were determined by measuring colony diameters after incubation on PDA plates from 30°C to 55°C with 5°C intervals for 24 to 48 h.

The range and optimal pH for fungal growth

The range of pH that thermophilic fungi could grow was determined by observing whether they could grow on PDA plates with pH values ranging from 3 to 12 after incubating for 24 to 96 h at their optimal temperature. The pH optima for fungal growth were determined by measuring colony diameters when PDA plates were adjusted to pH values from 5 to 10 and then incubated for 24 to 48 h at their optimal

temperature.

Fungal growth rate determination

Fungal growth rates were measured with the following equation when thermophilic strains were cultured at their optimal temperature and pH: $g=(d2 - d1)/24$, in which g represents the growth rate and $d1$ and $d2$ are the colony diameters measured at 24 and 48 h after inoculation (Redman *et al*., 1999).

Data analyses

The total number of thermophilic fungal isolates and their species identifications were recorded. For each species, the temperature and pH range were determined for all its isolates, including their minimum and maximum values recorded. The frequency of each thermophilic fungal species was calculated as a percentage of the total samples in which a particular species was registered. The relative abundance of each species was estimated as the number of particular species isolates in the sample series over the total number of isolates in the sample series.

Results

Culturable thermophilic fungi in geothermal soils

We obtained a total of 102 isolates of thermophilic fungi from geothermal soils in Tengchong Rehai National Park. On the basis of morphological features and ITS nucleotide sequence analyses, these strains were classified into 12 species (Table 1), including *Rhizomucor miehei* (9.80% of the total isolate number), *Chaetomium* sp. (5.88%), *Talaromyces thermophilus* (7.84%), *Talaromyces byssochlamydoides* (0.98%), *Thermoascus aurantiacus* Miehe var. *levisporus* (8.82%), *Thermomyces lanuginosus* (28.43%), *Scytalidium thermophilum* (22.55%), *Malbranchea flava* (4.90%), *Myceliophthora* sp. 1 (0.98%), *Myceliophthora* sp. 2 (3.92%), *Myceliophthora* sp. 3 (4.90%), and *Coprinopsis* sp. (0.98%). These species belonged to the fungal phyla Zygomycetes, Ascomycetes, Hyphomycetes, and Agonomycete. Among them, the species belongs to Agonomycete was identified as the anamorph of *Coprinopsis* depending on morphological and molecular investigations.

Phylogenetic analysis based on ITS nucleotide sequences

Using ITS5 and ITS4 primer pair, 500-700 bp rRNA gene containing the ITS I, ITS II and the intervening 5.8S rDNA region was amplified from all the thermophilic fungal isolates. The dendrogram based on ITS sequence analysis showed that the 12 species in our study were distributed in among most clades on the tree (Fig. 1). Each species in our study was clustered together with the same or the similar species that have been reported previously, and thus showed close genetic relatedness between them. Their close relatedness was also supported by morphological features (detailed morphological description in Table 2).

The range and optimal temperature for fungal growth

All of the thermophilic isolates had a minimum growth temperature at or above 25°C. A few strains in species such as *T. thermophilus* and *T. lanuginosus* could not grow below 35°C. On the other hand, all strains had a maximum growth temperature of at least 50°C, and some of them (e.g. *T. thermophilus*, *T. lanuginosus* as well as *Thermoascus aurantiacus* Miehe var. *levisporus*) even grew at 60°C. The optimal temperature for laboratory growth for the thermophilic strains ranged between 40°C and 50°C (Table 2).

The range and optimal pH for fungal growth

All of the thermophilic isolates had the capabilities to grow at a wide range of pH environments. Most strains could grow at pH 12 on the PDA agar plates. Some of them could grow at acidity conditions, but preferred alkalescent condition relatively. With regard to the optimal pH, most species grew best in the pH range between 7 and 8 (Table 2).

Fungal growth rate

All of the thermophilic fungi grew well on PDA plates at the optimal temperature and pH environments. Some of the strains grew very fast. For example, strains of *Rhizomucor*

Table 1. Thermophilic fungi from Tengchong Rehai National Park with their frequencies of occurrence and relative abundance

Species	Numbers	Sources	Sampling sites	Frequencies of occurrence (%)	Relative abundance (%)
Chaetomium sp.	6	4	Site 1	8.70	5.88
Coprinopsis sp.	1	1	Site 1	2.17	0.98
Malbranchea flava	5	4	Site 3	8.70	4.90
Myceliophthora sp. 1	1	1	Site 1	2.17	0.98
Myceliophthora sp. 2	4	2	Site 3	4.35	3.92
Myceliophthora sp. 3	5	5	Site 1, Site 3	10.87	4.90
Rhizomucor miehei	10	6	Site 1, Site 3	13.04	9.80
Scytalidium thermophilum	23	13	Site 1, Site 2, Site 3	28.26	22.55
Talaromyces byssochlamydoides	1	1	Site 1	2.17	0.98
Talaromyces thermophilus	8	7	Site 2, Site 3	15.22	7.84
Thermoascus aurantiacus Miehe var. *levisporus*	9	5	Site 3	10.87	8.82
Thermomyces lanuginosus	29	16	Site 1, Site 2, Site 3	34.78	28.43

Numbers represent the number of isolates; Sources represent the number of soil samples in which each species was registered; Frequencies of occurrence represent the percentage of the total samples in which a particular species was registered; Relative abundance represents the number of particular species isolates in the sample series over the total number of isolates in the sample series.

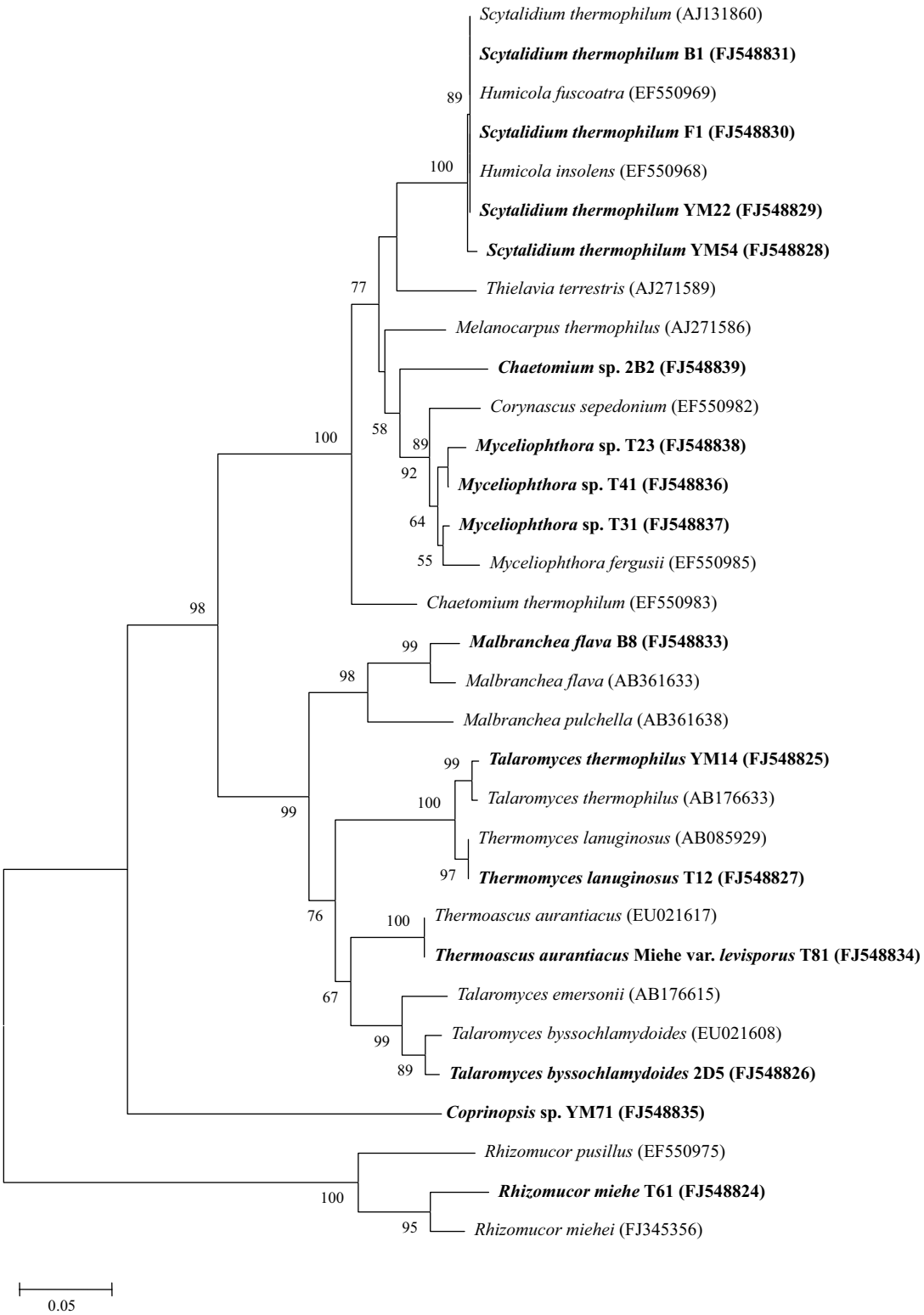

Fig. 1. ITS sequence-based phylogenetic tree of thermophilic fungi. The consensus NJ dendrogram with bootstrap values was constructed based on multiple sequence alignment using CLUSTAL X program. The strains isolated in our study are marked in bold.

Table 2. Summaries of physiological and morphological characteristics among all culturable thermophilic fungi from geothermal soils in Tengchong Rehai National Park

Species	Temperature range (°C)	Optimum temperature (°C)	pH range	Optimum pH	Growth rate (cm/day)	Representative strains number	Morphological characteristics	Accession no.
Chaetomium sp.	25-55	45	3-9	6-7	2.45±0.16	2B2	Brownish black colonies on PDA. Microscopic examination reveals wooly perithecium with terminal hairs.	FJ548839
Coprinopsis sp.	25-55	45	3-12	9	2.29±0.09	YM71	Slow growth on PDA, and the hypha is regularly provided with simple clamp connections at the transverse septa.	FJ548835
Malbranchea flava	30-55	40-45	4-12	7-8	0.91±0.08	B8	Cultured colonies show light yellow color with pinkish tinge and dark brown pigmentation on the reverse of the plate. The hypha shows typical coiled septate condiophores on the terminal portions.	FJ548833
Myceliophthora sp. 1	25-50	40	4-12	7	2.80±0.11	T23	Colonies on PDA cottony, zonate, irregular margins, yellowish orange, with yellowish orange exudate and yellowish orange at the reverse of agar plates.	FJ548838
Myceliophthora sp. 2	25-55	40	3-12	7-9	3.72±0.25	T31	Produces grayish brown colonies and brownish conidia conspicuously verrucose to spinulose.	FJ548837
Myceliophthora sp. 3	25-55	40	3-12	7-8	2.95±0.18	T41	Produces pinkish-cream floccose colonies, and aleuriospores are pyritorm to clavate, smooth and thick-walled, nearly hyaline.	FJ548836
Rhizomucor miehei	25-55	40	3-12	6-7	5.10±0.54	T61	Regularly produces zygospores in cultures, exhibits a looser sympodial branching pattern.	FJ548824
Scytalidium thermophilum[a]	25-55	45-50	5-12	8-9	2.05±0.19	YM54	Isolates have smooth-walled spores borne singly on short lateral branches with the absence of any pigmented spores.	FJ548828
Scytalidium thermophilum[b]	25-55	45	5-12	8	2.77±0.24	YM22	Aleuriospores are unicellular, bicellular or in chains of 3-4 spores, smooth, spherical or pyriform.	FJ548829
Scytalidium thermophilum[b]	25-55	45	5-12	9	4.10±0.31	F1	Regularly produces intercalary chlamydospores, in short chains in addition to solitary terminal spores on short lateral branches.	FJ548830
Scytalidium thermophilum[c]	25-55	40-45	5-12	8-9	2.65±0.33	B1	Isolates develop abundance intercalary, slightly pigmented spores in chains.	FJ548831
Talaromyces byssochlamydoides	30-55	40-45	3-8	6	2.53±0.06	2D5	Distinguished by its conspicuous *Paecilomyces* anamorph. Ascomata always develops in culture concomitantly with the anamorph.	FJ548826
Talaromyces thermophilus	35-60	45-50	4-12	7-8	1.03±0.20	YM14	Characterized to have a Penicillium anamorphic state producing green conidia. Ascospores are ellipsoidal, ornamented by 2-6 somewhat jagged, irregular ridges.	FJ548825
Thermoascus aurantiacus Miehe var. *levisporus*	30-60	50	3-11	7	8.91±0.72	T81	Typically golden orange colored colonies and reverse of the plate are brownish. Presence of terminal aleuriospores apparent, ascospores are elliptical and smooth.	FJ548834
Thermomyces lanuginosus	35-60	50	4-12	7-8	1.33±0.35	T12	Culture with greenish grey to purple brown color and typical wine red pigmentation at the reverse of agar plates. Presence of wrinkled phialospores in culture.	FJ548827

[a] and [c] represent the type 1 isolates and type 2 isolates of *Scytalidium thermophilum*
[b] represent the identified isolates which were intermediate between types 1 and 2 (detailed description in Discussion)

miehei and *Thermoascus aurantiacus* Miehe var. *levisporus* reached 5.10±0.54 cm/day and 8.91±0.72 cm/day, respectively. The growth rate results are summarized in Table 2.

Discussion

As was proposed by Jean Mouchacca (1997), optimum growth at temperatures above the maximum threshold of mesophiles characterize few Mucorales, Eurotiales, and Sphaeriales, a limited number of Hyphomycetes, and one Agonomycete species. In 2000, Maheshwari *et al*. (2000) proposed a list of 28 common thermophilic fungal species. In our investigations, the thermophilic fungi from geothermal soils in Tengchong Rehai National Park contain 12 species, including one species of the Mucorales (*R. miehei*), one specie of the Sphaeriales (*Chaetomium* sp.), three species of the Eurotiales (*Thermoascus aurantiacus* Miehe var. *levisporus*, *T. byssochlamydoides*, and *T. thermophilus*), six species of the Hyphomycetes (*T. lanuginosus*, *S. thermophilum*, *M. flava*, *Myceliophthora* sp. 1, *Myceliophthora* sp. 2, and *Myceliophthora* sp. 3), and one species of Agonomycete (*Coprinopsis* sp.). These 12 species represent approximately half of the common thermophilic fungi recorded so far, consistent with an extremely high diversity of thermophilic fungi in this area.

As shown in Table 1, another feature of the thermophilic fungi from Tengchong Rehai National Park was that strains of *T. lanuginosus* and *S. thermophilum* were very prevalent. Both species were found in all three sampling sites, with the frequencies of occurrence at 34.78% and 28.26%, respectively. *T. lanuginosus* (formerly known as *Humicola lanuginosa*) occurs worldwide and has been isolated in many countries from a variety of habitats, including dry and waterlogged grasslands, loamy garden soils and aquatic sediments (Singh *et al*., 2003). It has also been isolated from the air in Indonesia and the British Isles (Hudson, 1992). The broad temperature and pH ranges of *T. lanuginosus* strains (Table 2) may be related to its wide adaptation to different habitats. *S. thermophilum* is one of the important thermophilic fungi in compost (Straatsma and Samson, 1993). According to previous studies, this fungus represents the *Torula-Humicola* complex which contained isolates assigned to *S. thermophilum*, *Torula thermophila*, *Humicola insolens*, and *Humicola grisea* var. *thermoidea* (Ellis and Griffiths, 1976). In this species, it has been shown that there are two extreme cultural types (Straatsma and Samson, 1993; Lyons *et al*., 2000): type 1 isolates had single very dark spores borne on short lateral hyphal branches and were designated as *H. grisea* var. *thermoidea* type; type 2 isolates developed intercalary, slightly pigmented spores in chains, typical of *S. thermophilum*. Additionally, there is another type of isolates that were intermediate between types 1 and 2. All of the cultural types of *S. thermophilum* occurred in our isolates (Table 2). Contrarily, other species with low abundance and frequency of occurrence were also isolated in this area. Three of them (*T. byssochlamydoides*, *Myceliophthora* sp. 1, and *Coprinopsis* sp.) were represented only by one isolate each and they have not been isolated in other geothermal environments.

In 1999, Redman *et al*. (1999) characterized fungal communities in geothermal soils around a sulfurous hot spring in Yellowstone National Park, where they isolated 2 thermophilic fungal species. In 2003, a similar investigation was done in Northern Taiwan by Chen *et al*. (2003). In that research, 7 of the 12 species of fungi from sulfurous hot spring soils were thermophilic. Furthermore, their fungal community was simple and consisted completely of species of Deuteromycetes (most *Aspergillus fumigatus* complex). Compared with those reports, the thermophilic fungi from Tengchong Rehai National Park are more diversity (12 species). Such a high diversity might be due to the abundant nutrition in the geothermal soils around neutral and alkalescent thermal springs, which can be also supported by the high diversity of plants observed in this area. Furthermore, the stressful and selective conditions of the neutral and alkalescent thermal springs compel the thermophilic fungi in this area to thrive at alkaline conditions as it has partially been confirmed by our assays of pH range for fungal growth.

Based on our data, the thermophilic fungi from geothermal soils around neutral and alkalescent thermal springs are more diversity and possess relatively strong capability to tolerate the alkalescent environment in Tenchong Rehai National Park. Therefore the application of the thermophilic fungi resources from this region is expected, and thus worth in-depth study.

Acknowledgements

The authors wish to thank Prof. HanBo Zhang for his helpful comment on the manuscript. In addition, we thank Dr. Jianping Xu (McMaster University, Canada) for English revisions. The research described here is supported by the Department of Science and Technology of Yunnan Province, China (grant No. 2006PY01-27).

References

Aguilar, A. 1996. Extremophile research in the European Union: from fundamental aspects to industrial expectations. *FEMS Microbiol. Rev.* 18, 89-92.

Apinis, A.E. 1967. Dactylomyces and Thermoascus. *Trans. Br. Mycol. Soc.* 30, 573-582.

Bao, Q., Y. Tian, W. Li, Z. Xu, Z. Xuan, S. Hu, W. Dong, and *et al*. 2002. A complete sequence of the *T. tengcongensis* genome. *Genome Res*. 12, 689-700.

Barns, S.M., C.F. Delwiche, J.D. Palmer, and N.R. Pace. 1996. Perspectives on archaeal diversity, thermophily, and monophyly from environmental rRNA sequences. *Proc. Natl. Acad. Sci. USA* 93, 9188-9193.

Bruns, T.D., T.J. White, and J.W. Taylor. 1991. Fungal molecular systematics. *Annu. Rev. Ecol. Syst*. 22, 525-564.

Chen, K.Y. and Z.C. Chen. 1996. A new species of *Thermoascus* with a *Paecilomyces* anamorph and other thermophilic species from Taiwan. *Mycotaxon* 50, 225-240.

Chen, K.Y., D.J. Huang, and C.C. Liu. 2003. The mycoflora of hot spring soil in northern Taiwan. *Taiwania* 48, 203-211.

Chen, C., L. Lin, Q. Peng, K. Ben, and Z. Zhou. 2002. *Meiothermus rosaceus* sp. nov., isolated from Tengchong hot spring in Yunnan, China. *FEMS Microbiol. Lett.* 216, 263-268.

Cooney, D.G. and R. Emerson. 1964. Thermophilic fungi: an account of their biology, activities, and classification. W. H. Freeman and Co., San Francisco, USA.

Ellis, D.H. 1981. Ultrastructure of thermophilic fungi IV. Conidial ontogeny in *Thermomyces*. *Trans. Br. Mycol. Soc.* 77, 229-241.

Ellis, D.H. 1982. Ultrastructure of thermophilic fungi V. Conidial ontogeny in *Humicola grisea* var. *thermoidea* and *H. insolens*. *Trans.*

Br. Mycol. Soc. 78, 129-139.

Ellis, D.H. and D.A. Griffiths. 1976. The fine structure of conidial development in the genus *Torula*. IV. *T. thermophila* Cooney & Emerson. *Can. J. Microbiol.* 22, 1102-1112.

Gené, J., J.M. Guillamón, J. Guarro, I. Pujol, and K. Ulfig. 1996. Molecular characterization, relatedness and antifungal susceptibility of the basidiomycetous *Hormographiella* species and *Coprinus cinereus* from clinical and environmental sources. *Antonie van Leewenhoek* 70, 49-57.

Guarro, J., S.K. Abdullah, S.M. Al-Bader, M.J. Figueras, and J. Gene. 1996. The genus *Melanocarpus*. *Mycol. Res.* 100, 75-78.

Hudson, H.J. 1992. Fungal Biology, pp. 106-170. Cambridge University Press, Cambridge, UK.

Hugenholtz, P., C. Pitulle, K.L. Hershberger, and N.R. Pace. 1998. Novel division level bacterial diversity in a Yellowstone hot spring. *J. Bacteriol.* 180, 366-376.

Lin, L., C. Chen, Q. Peng, K. Ben, and Z. Zhou. 2002. *Thermus rehai* sp. nov., isolated from Rehai of Tengchong, Yunnan Province, China. *J. Basic Microbiol.* 42, 337-344.

Lin, L., J. Zhang, Y. Wei, C. Chen, and Q. Peng. 2005. Phylogenetic analysis of several *Thermus* strains from Rehai of Tengchong, Yunnan, China. *Can. J. Microbiol*. 51, 881-886.

Lyons, G.A., G.J. Mckay, and H.S.S. Sharma. 2000. Molecular comparison of *Scytalidium thermophilum* isolates using RAPD and ITS nucleotide sequence analyses. *Mycol. Res*. 104, 1431-1438.

Maheshwari, R., G. Bharadwaj, and M.K. Bhat. 2000. Thermophilic fungi: Their physiology and enzymes. *Microbiol. Mol. Biol.* R. 64, 461-488.

Millner, P.D., J.J. Motta, and P.L. Lentz. 1977. Ascospores, germ pores, ultrastructure, and thermophilism in *Chaetomium*. *Mycoloigia* 69, 720-733.

Mouchacca, J. 1997. Thermophilic fungi: biodiversity and taxonomic status. *Cryptogamie Mycol.* 18, 19-69.

Redman, R.S., A. Litvintseva, K.B. Sheehan, J.M. Henson, and R.J. Rodriguez. 1999. Fungi from geothermal soils in Yellowstone National Park. *Appl. Environ. Microbiol*. 65, 5193-5197.

Schipper, M.A.A. 1978. On the genera *Rhizomucor* and *Parasitella*. *Studies Mycol*. 17, 53-71.

Schwarz, P., S. Bretagne, J.C. Gantier, D. Garcia-Hermoso, O. Lortholary, F. Dromer, and E. Dannaoui. 2006. Molecular identification of Zygomycetes from culture and experimentally infected tissues. *J. Clin. Microbiol.* 44, 340-349.

Sharma, M., B.S. Chadha, M. Kaur, S.K. Ghatora, and H.S. Saini. 2008. Molecular characterization of multiple xylanase producing thermophilic/thermotolerant fungi isolated from composting materials. *Lett. Appl. Microbiol.* 46, 526-535.

Singh, S., A.M. Madlala, and B.A. Prior. 2003. *Thermomyces lanuginosus*: Properties of strains and their hemicellulases. *FEMS Microbiol. Rev.* 27, 3-16.

Stetter, K.O. 1999. Extremophiles and their adaptation to hot environments. *FEBS Lett.* 452, 22-25.

Straatsma, G. and R.A. Samson. 1993. Taxonomy of *Scytalidium thermophilum*, an important thermophilic fungus in mushroom compost. *Mycol. Res.* 97, 321-328.

Upadhyay, J.M., M.S. Farmelo, S.G. Goetz, and M.A. Melan. 1984. A new variety of a thermophilic mold, *Thermoascus aurantiacus* var. *levisporus*. *Mycopathologia* 87, 71-80.

Van Oorschot, C.A.N. 1977. The genus *Myceliophthora*. *Persoonia* 9, 401-408.

Voigt, K., E. Cigelnik, and K. O'donnell. 1999. Phylogeny and PCR identification of clinically important Zygomycetes based on nuclear ribosomal-DNA sequence data. *J. Clin. Microbiol.* 37, 3957-3964.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315-322. *In* M.A. Innis, D.H. Gelfand, and J.J. Sninsky (eds.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA, USA.