

## Thermotolerant and Thermoresistant *Paecilomyces* and its Teleomorphic States Isolated from Thai Forest and Mountain Soils

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### ABSTRACT

A Dilution plate method combined with heat treatment at 60°C and 80°C was used to isolate thermotolerant and thermoresistant *Paecilomyces* species in soil. The predominant species of *Paecilomyces* that had been identified was *Paecilomyces variotii*, the type species of the genus. Oatmeal Agar was used to induce the teleomorph at 37°C. Other species isolated belong to *Paecilomyces* or its teleomorphic states *Byssochlamys*, *Talaromyces* and *Thermoascus* included *Byssochlamys nivea*, *Byssochlamys fulva*, *Talaromyces byssochlamydoides* and *Thermoascus crustaceus*.

**Key words:** soil fungi, *Paecilomyces*, *Byssochlamys*, *Talaromyces*, *Thermoascus*, thermotolerant, thermoresistant

### INTRODUCTION

Fungi are the most abundant component of the soil microflora in terms of biomass. They can be divided into three general functional groups based on how they get their energy (Gams *et al.*, 1998). As *decomposers* – Fungi are the major decomposers (saprobic fungi) in the soil, especially in forest soils, mainly participating in cellulose, chitin and lignin decomposition. As *mutualists* – Mycorrhizal fungi colonize plant roots helping the plant to solubilize phosphorus and bring soil nutrients to the plant in exchange for carbon. And as *parasites/pathogens* - This third group of fungi causes reduced production, disease or death when they colonize roots and other organisms. Fungi are present in the soil as mycelium, conidia, ascospores,

chlamydospores or sclerotial bodies. With the exception of the mycelium that may have little metabolic activity, the mentioned stages are all dormant survival structures, having little activity and limited importance in the metabolism of the soil.

Thermophilic fungi are of economic importance with several reported contaminants of food products. Because of their thermophily the species can also grow above the body temperature of higher animals hence are potential human pathogens. Importantly, thermophilic fungi are a potential source of thermotolerant enzymes (Jahri *et al.*, 1999; de Koe *et al.*, 2000). There are currently ca. 40 species of thermophilic fungi known, belonging to a wide array of 21 genera (Mouchacca, 1999).

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The genus *Paecilomyces* comprises many saprobic species commonly isolated from soil but is also found in a wide range of substrata, such as plants, animals, food products, air and indoor environment, and also from insects. Pitt and Hocking (1985) considered *Paecilomyces* to be a species interface between *Aspergillus* and *Penicillium*. The genera *Paecilomyces*, *Aspergillus* and *Penicillium* are recognized as prime sources of novel metabolites and are of recognized importance to industry (Samson *et al.*, 2000 ; Scott, 1994).

*Paecilomyces variotii*, the type species of Section *Paecilomyces*, a thermophilic fungus most commonly isolated from hay, is probably one of the most common species of *Paecilomyces sensu lato* which was first described by Bainier (1907). The genus is worldwide in its distribution, showing considerable morphological heterogeneity. The latest monographic treatment to the genus was by Samson (1974), where he included species isolated from insects. In this monograph, *Paecilomyces* was divided into two sections: Section *Paecilomyces* containing mostly thermotolerant to thermophilic taxa and Section *Isarioidea* containing mostly mesophilic species isolated from insects. Section *Paecilomyces* has teleomorphs in the genera *Talaromyces*, *Byssochlamys*, *Aphanoascus* and *Thermoascus*.

There are nine species belonging to Section *Paecilomyces* according to Samson (1974): *Paecilomyces variotii* (type species), *Paecilomyces aerugineus*, *Paecilomyces clavisporus*, *Paecilomyces fulvus*, *Paecilomyces niveus*, *Paecilomyces zollerniae*, *Paecilomyces leycettanus*, *Paecilomyces byssochlamydoides* and *Paecilomyces crustaceus*. Out of these nine species there were three known teleomorphs: *Byssochlamys*, *Talaromyces* and *Thermoascus*. The purpose of this study was to determine the number of *Paecilomyces* species of section *Paecilomyces* found in Thai soils.

## MATERIALS AND METHODS

Soil from different forest and mountainous locations in Thailand were collected and investigated between January 2001- April 2002. These sites covered mountain areas in Saraburi, Lopburi and Chiang Mai mountains as well as the following national parks and wildlife sanctuaries: Doi Inthanon National Park, Khao Yai National Park, Hala Bala Wildlife Sanctuary, Pha Pru Sirindorn, Erawan National Park. The dilution plate technique (Koch'sches Plattengußverfahren) was applied for isolation of soil fungi. This technique was combined with heat treatment of the soil to be selective only for thermoresistant organisms. To be selective only for fungi, antibiotics were added to the media.

*Procedure.* Soil diluted with sterile water 1:10 (w/v) was shaken by hand for 10 minutes. A 30-minute heat treatment was done at 60°C and 80°C for isolation of thermoresistant fungi. The suspension was then furtherly diluted ( $10^{-4}$  to  $10^{-9}$ ). 1 ml aliquots of these dilutions were pipetted onto the agar plates (2% Malt Extract Agar, Difco) containing antibiotics (Penicillin, 50 ppm and Streptomycin, 50 ppm) and were distributed evenly with a glass rod. The plates were then incubated at 37°C. Colony growth of fungi was checked under the microscope daily for three days. Colonies were picked and transferred onto fresh agar plates and were furtherly incubated at 37°C for identification.

A key produced by Samson (1974) was used for identification of *Paecilomyces*. Slide preparations of cultures grown on 2% MEA at 37°C were made and examined under the light microscope for identification. All suspected *Paecilomyces* species were grown separately into Oatmeal Agar (OA) to induce teleomorph production. Incubation temperature was also at 37°C. Pure cultures were grown on a sterile filter paper placed on top of 2% MEA. After the filter paper was fully-grown with fungi, it was removed from the agar plate and transferred to a sterile glass

plate and placed in a dessicator for two weeks. The dried filter paper was then put in sterile aluminum foil sheets and sealed airtight in a plastic bag. This was stored at -20°C.

## RESULTS AND DISCUSSION

Of the nine *Paecilomyces* species found under Section *Paecilomyces*, only five species were identified and isolated in Thailand using dilution plate technique. These were: *Paecilomyces variotii*, *Byssochlamys fulva* (*P. fulvus*), *Byssochlamys nivea* (*P. niveus*), *Talaromyces byssochlamydoides* (*P. byssochlamydoides*) and *Thermoascus crustaceus* (*P. crustaceus*).

### *Paecilomyces variotii* Bainier

Teleomorph: *Talaromyces spectabilis*  
Udagawa and Suzuki

Strains\*<sup>1</sup>: TR 59, TR 82, TR 85, TR 92, TR 195, T 123, T 127, T 129, T 172, T 226, T 234, T 268, T 269, T 364, T 180, T 343, T 347, T 404, T 424

*Paecilomyces variotii* Bainier was the most common among the species isolated, comprising both thermotolerant and thermoresistant strains. This species was present in almost every soil sampled. Although Samson (1974) broadened the definition of the species and incorporated deviating strains, there were slight morphological differences in the colony growth of *P. variotii* cultures. The texture of the colonies varied from funiculose to velvety. Variations in the color of the colony were observed ranging from light yellow to olive brown. Figure 1 shows the variation of colony texture and colors of selected Thai isolates. Growth was very rapid on both 2% MEA and OA. Sporulation occurred after one day of incubation. Cultures grown on OA failed to produce the sexual state. All strains examined under the microscope

produced chlamydo spores except for two strains (TR 59 and TR 82). The sizes of the chlamydo spores were almost all in the same ranges (4-8 µm). Conidia globose, subglobose to ellipsoidal, color mostly yellow, yellow-brown in mass turned to olive brown with age. Size of conidia varied from 2-3 3-6 µm. Phialides either in whorls, solitary or intercalary varied from 6-13×2-3µm consisting of inflated basal portion and tapers into a long neck, usually 1 µm in diameter.

The color variations raise the question of whether we are dealing with color varieties or truly distinct species in a complex. Further studies, especially molecular or chemical, are recommended to see if the differences observed in the colony texture and especially color are due to media, temperature or cultural influences. Only then can conclusions be made as to whether *Paecilomyces variotii* is indeed one species or an aggregate of several.

In Thailand *Paecilomyces variotii* has been previously reported from soil of mixed deciduous forest in Huay Kha Khang Wildlife Sanctuary, Uthai Thani province (Manoch *et al.*, 2000), a termite mound in Chiang Mai province (Manoch *et al.*, 2004), agricultural soil (Supro *et al.*, 1999) and even from stream water of a teak plantation in Kanchanaburi province (Kosol *et al.*, 1999). Worldwide, *P. variotii* is a rather common fungus in the soil, in air, in self-heated substrates, in compost, on wood and is frequently recorded as a moderately thermophilous organism (Samson, 1974; Domsch *et al.* (1993)). It has also been reported in food and food products, such as cocoa beans, rice-flour, fruit juices and from animal feeding stuffs.

### *Byssochlamys fulva* Olliver and G. Smith

Anamorph: *Paecilomyces fulvus* Stolk & Samson

<sup>1</sup> T represents strains isolated with heat-treatment at 60°C for 30 minutes, TR represent strains using heat-treatment at 80°C for 30 minutes.

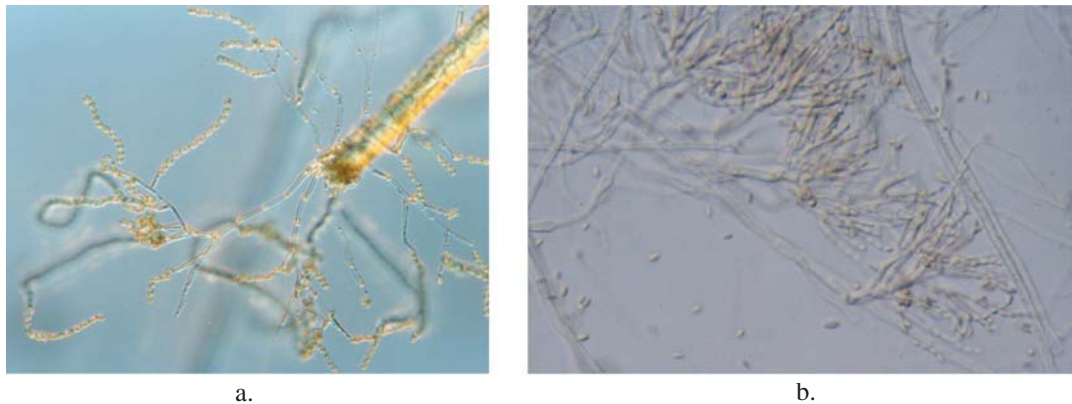
Strains: T 152, T 332, T 333, T 363, T 382

Five strains of *Byssochlamys fulva* were isolated from forest soil in the south of Thailand (Pha Pru Sirindorn), Suan Pueksasaat in Chiang Mai and from a termite mound in Chiang Mai and Doi Suthep (Chiang Mai). Manoch *et al.* (2004)

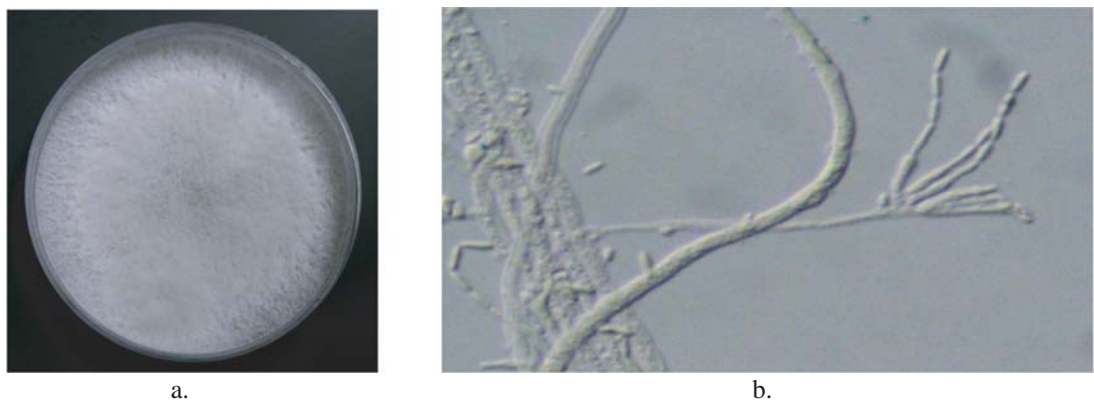
reported this species from termite mounds in Saraburi and Mae Hong Son. The shapes of the conidia of the anamorphic state, the size of ascospores and the presence or absence of chlamydospores were the distinguishing characteristics between *B. fulva* and *B. nivea*. This



**Figure 1** Textures and colors of thermotolerant *P. variotii* colonies on 2% MEA. From left to right: T 172, T 226, T 234 and T 268.



**Figure 2** *Paecilomyces variotii* under light microscope. a. conidiophores, b. phialides and conidia.

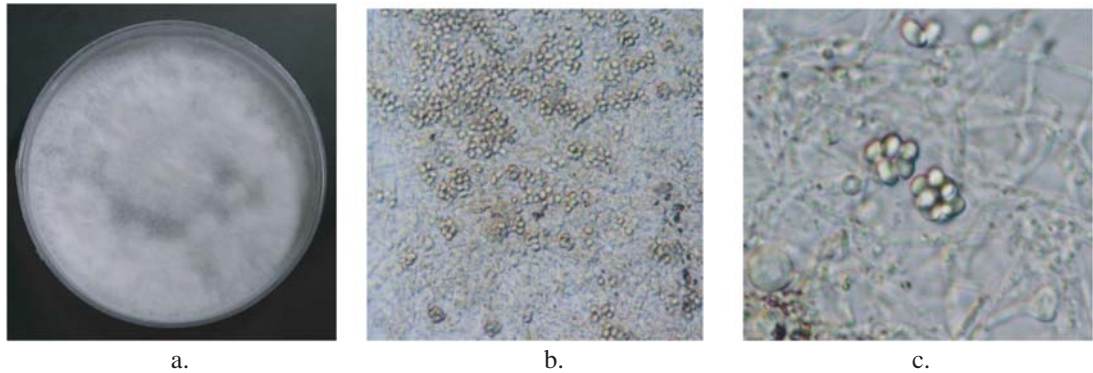


**Figure 3** *Byssochlamys fulva* a. colony appearance, b. conidiophore and conidia.

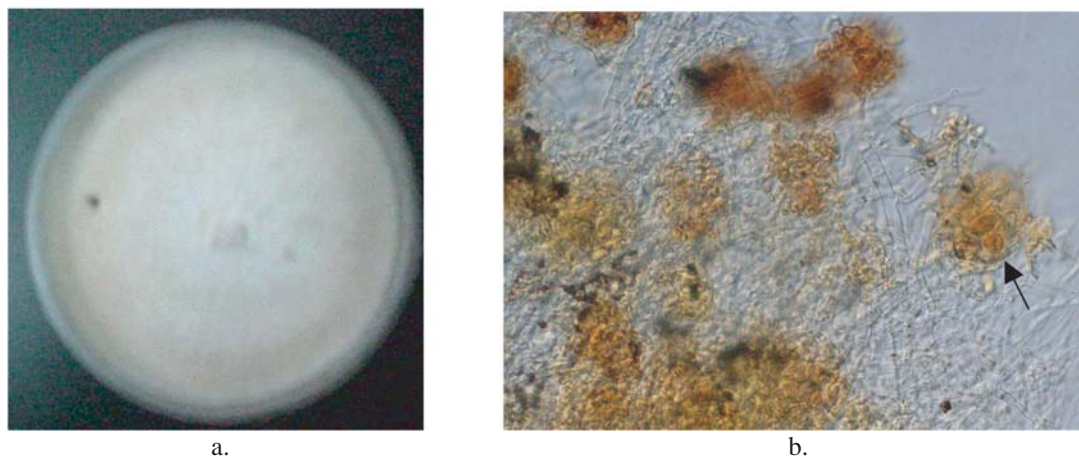


species produced a *Paecilomyces* anamorph with smooth-walled elongated conidia having blunt ends, yellow,  $4-8 \times 2-5 \mu\text{m}$ . Ascospores  $5.2-6.5 \times 3.4-4.0 \mu\text{m}$ . Chlamydospores were absent.

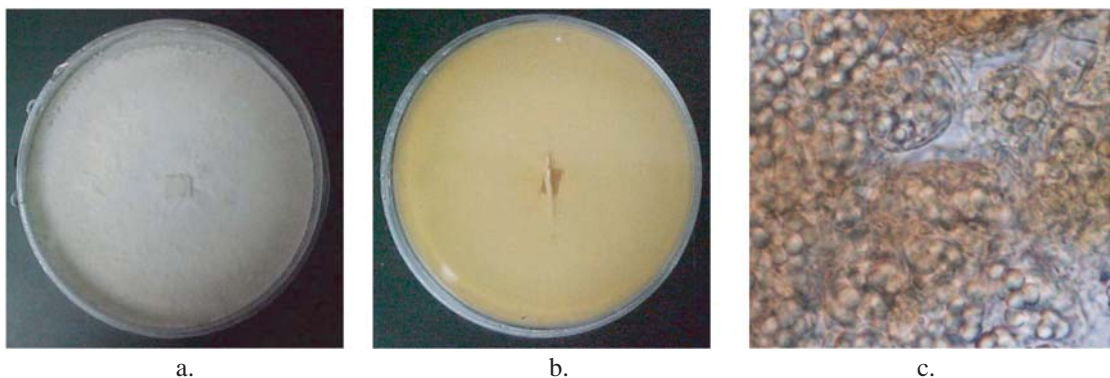
*Byssochlamys fulva* produces a range of pectolytic enzymes, including polygalacturonase, polymethylgalacturonase and pectate lyase (Chu and Chang, 1973) including a large amounts of



**Figure 4** *Byssochlamys nivea* a. colony appearance b, c. ascomata and ascospores.



**Figure 5** *Talaromyces byssochlamydoides*. a. colony appearance, b. ascospore formation.



**Figure 6** *Thermoascus crustaceus* colony appearance. a. obverse b. reverse c. ascospores.

byssochlamic acid, a moderately toxic compound which can inhibit the growth of *Sinapsis alba* seedlings and the fermentation of the yeast *Saccharomyces cerevisiae* (Meyer and Rehm, 1967 and 1969). Experiments in mice and guinea pigs caused toxic effects to the liver (Raistrick and Smith, 1933 ; Gedek, 1971).

### ***Byssochlamys nivea* Westling**

Anamorph: *Paecilomyces niveus* Stolk and Samson

Strains: T 135, T150, T 153

Three strains of *Byssochlamys nivea* were isolated from Lopburi and Pha Pru Sirindorn. Variations in the colony morphology and size of ascospores were observed, probably hinting varieties within this species. The *Paecilomyces* anamorph produced globose to subglobose conidia,  $3-6 \times 2-4 \mu\text{m}$ , and all strains produced chlamydo-spores (up to  $10 \mu\text{m}$  in diameter, thick-walled, smooth walled, globose to ovoid) abundantly in culture. A previous report of *B. nivea* from Thailand was from a termite mound in Sakolnakorn province (Manoch *et al.*, 2004).

*Byssochlamys nivea* and *B. fulva* have heat-resistant ascospores and are important contaminants of canned fruit and fruit juices (Eckhardt and Ahrens, 1977). The ascospores can survive exposure to the temperatures of 80 and 90°C for 10 minutes (King *et al.*, 1969; Partsch *et al.*, 1969). Both species are also able to produce mycotoxins (e.g. byssochlamic acid, patulin) (Samson *et al.*, 2002).

### ***Talaromyces byssochlamydoides* Stolk and Samson**

Anamorph: *Paecilomyces byssochlamydoides* Stolk and Samson

Strains: T 212

Only one strain of *Talaromyces byssochlamydoides* was identified and isolated from Erawan National Park, Kanchanaburi. Kanjanamaneesathian (1988) made the first report

on this species from agricultural soils in Prachuap Khiri Khan. The colony produced orange-colored ascomata, which ripened after a few days on incubation at 37°C. Conidia were yellow, ellipsoidal to cylindrical in shape ranging from  $4-8 \mu\text{m} \times 1-2.5 \mu\text{m}$  and rounded at the ends. The conidia of the *Paecilomyces* anamorph showed some resemblance to conidia of *Thermoascus crustaceus*. Stolk and Samson (1972) reported that *I. byssochlamydoides* is strongly thermophilic, minimum temperature at 25°C, optimum 40-45°C, maximum slightly above 50°C.

### ***Thermoascus crustaceus* (Apinis and Chesters) Stolk**

Anamorph: *Paecilomyces crustaceus* Apinis and Chesters

Strains: T 157

This was the first record of this teleomorph in Thailand. The *Paecilomyces* anamorph was also found. One strain of *Thermoascus crustaceus* was isolated from Khao Yai National Park. The ascospores were oval,  $6.5-8 \times 5-6.5 \mu\text{m}$ . Samson (1974) mentioned that this species was a very common thermophilic organism. From Thai forest soils, however, it was rather rare. Cruesrisawath (1985) first reported *Thermoascus aurantiacus* from soil, debris and straw in Chantaburi after incubating the materials at 45°C. Two species of *Thermoascus* has also been reported from Thailand (Kanjanamaneesathian, 1988): *Thermoascus aurantiacus* and a *Thermoascus* sp. from cultivated soil in Prachuap Khiri Khan province. These two species were thermophilic and grew well at 45°C.

Little is known about the toxins, secondary metabolites and enzymes produced by *Thermoascus crustaceus* and *Talaromyces byssochlamydoides*. These two species are thermophilic and could be potentially good producers of interesting thermophilic enzymes and secondary metabolites. It has not been reported as food contaminants or pathogens.

Most of the soils sampled for the isolation

of *Paecilomyces* species in this study were from the sun-heated areas of the Thai forest. Although soil is a very good habitat for thermophilic fungi, variation in the abundance of individual species can depend on the type of soil, depth, season of the year, concentration of organic materials and isolation technique employed (Subrahmanyam, 1999). It is recommended that for better results different kinds of soil (garden, street rand, riverbank, garbage area, composts, etc) and at different seasons should be sampled for heat-resistant and heat-tolerant *Paecilomyces*. So far, only five species have been identified using dilution plate method coupled with heat-treatment.

### CONCLUSIONS

Out of the five *Paecilomyces* species isolated from Thai forest soils, *Paecilomyces variotii*, the type species, was the most commonly encountered. Other species belonging to *Paecilomyces* Section *Paecilomyces* identified were: *Byssoschlamys fulva*, *Byssoschlamys nivea*, *Talaromyces byssoschlamydoidea* and *Thermoascus crustaceus*. These four other species were not so common in the forest and mountain soils. This is the first report of *Thermoascus crustaceus* in Thailand.

### ACKNOWLEDGMENTS

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