A new thermotolerant *Paecilomyces* species which produces laccase and a biform sporogenous structure

Zong-Qi Liang[1*], Yan-Feng Han[1] and Hua-Li Chu[2]

[1]Institute of Fungus Resources, Guizhou University, Guiyang 550025, PR China
[2]Hainan University, Haikou 570228, PR China

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A new thermotolerant species, *Paecilomyces biformis* isolated from a soil sample collected in Tengchong Geothermal National Geopark, Yunnan Province, PR China is described and illustrated. This fungus can be easily distinguished from the other species of the genus *Paecilomyces* by the biform sporogenous structure consisting of phialides that are borne singly and finite conidiophores, finely echinulate stalks of the conidiophores and large conidia. On PDA plates containing different concentrations of O-methoxyphenol, the fungus exhibited laccase activities. A key to the accepted thermotolerant species of *Paecilomyces* is provided.

Key words: laccase, morphology, *Paecilomyces*, *Paecilomyces biformis* taxonomy, thermotolerant fungus

Introduction

Brown and Smith (1957) in their study on the genus *Paecilomyces* at first introduced one monophialidic species, *P. flavescens* A.H.S. Br. & G. Sm. [= *P. inflatus* (Burnside) J.W. Carmich.].

Onions and Barron (1967) transferred 10 monophialidic species, including *P. inflatus* as a member, to the genus *Paecilomyces*. The main conidiophore axes of these species are lacking and have orthotropic awl-shaped phialides. In these forms, the phialides are borne singly either directly on the vegetative hyphae or often in groups of two or three on very short conidiophores. For convenience, they grouped these species together as a monophialidic series within the genus *Paecilomyces* as presented by Brown and Smith (1957).

Gams (1971) transferred other awl-shaped monophialidic species to the genus *Acremonium* and left only *P. inflatus* in the genus *Paecilomyces* because the phialides are flask-shaped and have somewhat inflated basal

*Corresponding author: Z.Q. Liang; email: zqliang472gzu@yahoo.com.cn

portion and sometimes cluster in small groups on well-defined very short conidiophores. Subramanian (1972) placed some of these species together with *Gliomastix murorum* in a new genus, *Sagrahamala.* Since then only several other species, *P. ampullaris* Matsush. (Matushima, 1971), *P. ampulliphoris* Matsush., *P. iriomoteanus* Matsush. (Matushima, 1975) and several thermotolerant and laccase-producing new taxa, *P. major* (Z.Q. Liang, H.L. Chu & Y.F. Han) Z.Q. Liang, H.L. Chu & Y.F. Han (Chu *et al.*, 2004; Liang *et al.*, 2006a), *P. furcatus* Z.Q. Liang, H.L. Chu & Y.F. Han, *P. cinereus* Z.Q. Liang, H.L. Chu & Y.F. Han (Liang *et al.*, 2006b) and *P. curticatenatus* Z.Q. Liang & Y.F. Han (Han *et al.*, 2007) were reported from China.

In this paper, we report another new thermotolerant species of *Paecilomyces* with a biform sporogenous structure and producing laccase.

Materials and methods

Collection of strain and isolation

The strain studied GZDXIFR-H28 was isolated from a soil sample of Tengchong Geothermal National Geopark, Yunnan Province, PR China, July, 2004. Two grams of soil sample were added into a flask containing 20ml sterilized water and glass balls. The soil suspension was diluted into a concentration of 10^{-1}-10^{-2} after shaking for about 10min. One ml of soil suspension at concentration of 10^{-2} was mixed with Martin medium in one 9 cm diameter Petri dish sterilized, then incubated at 40ºC in an airproofed thermotolerant plastic bag for about 4 days. Colonies showing sporogenous structure of *Paecilomyces* were purified by transplanting onto Martin's slants.

Identification for strains

The strains were transplanted onto Czapek agar, potato dextrose agar (PDA), and Sabouraud agar in accordance with procedures set forth in Brown and Smith (1957) and Samson (1974). After incubation at 40ºC for 7 days, strains were identified based on colony characters, sporogenous structures, and other biological features.

The type strain of *P. biformis* GZDXIFR-H28 and the holotype GZDXIFR-H28-1, a dried plate culture on Czapek agar, were deposited in the Institute of Fungus Resources, Guizhou University, China. The sequence of rDNA ITS1-5.8s-ITS2 region of this fungus has been submitted to GenBank (accession number: DQ191963).

Examination of laccase activities

In PDA plates, 0.02%, 0.03%, 0.04% and 0.05% O-methoxyphenol were added, respectively, as substrate referring to the method for screening microbes of laccase-producing (Wang and Zhu, 2003).

For detecting enzyme activity, a diameter of 7 mm disc inoculation for 4 days incubation, was transplanted to the centre of a PDA plate containing O-methoxyphenol of different concentrations, respectively. Then these plates were placed in an attemperator, at 40ºC, and the oxidation zone of laccase was examined momentarily. *Paecilomyces* sp. GZDXIFR- H104-2, a strain without enzyme activity, was used as the check. All analyses were carried out in triplicate to minimize errors.

Results and Discussion

Description and identification of the new species

Paecilomyces biformis Z.Q. Liang, H.L. Chu & Y.F. Han, **sp. nov.**

(Figs 1-12)

MycoBank: 510977

Etymology: latin. *biformis*, referring to biform of sporogenous structure.

In agaro Czapekii, *coloniae* 65 mm diam in 7 diebus ad 40ºC, griseolae, velutinae brivae, zonis brunnescentibus prope margibus; reversum nigrum. *Hyphis* 1-5.3 µm crassis. *Structurae* sporogenae bi- formatae, A-forma: *phialides* singulares, 5-22.3 (-25.3) × (1.5-)1.9-5.5 (-6.2) µm, e basi inflata, ovoidea, ellipsoidea vel cylindrica in collum distinctum apice inspissato angustatae, aliquot prolificae. B-forma: *conidiophora* erecta, mononematicea, e hyphis submerses vel raro lateraliter e hyphis aeries oriuntur, in apce phialides. *Conidia* levia, fusiformia vel longi-ellipsoidea, (3.5-) 4.5-10.4 (-13) × (-2) 3.5-5 (-7) µm.

Typus: GZDXIFR-H28 et cultara viva, isolatus GZDXIFR-H28-1 e solo, Tengchong, Provinica Yunnan, VII, 2004, Chu H. L.; in Guizhou Univ., conservatur.

Colonies on Czapek agar usually growing rapidly, attaining a diameter of 65 mm within 7 days at 40ºC; rotundity, flat, short velvety, gray, pale brown zones in margin; reverse dark, gray in margin. *Vegetative hyphae* septate, hyaline, smooth-walled, 15.3 µm wide. Sporogenous structure biform: *A-sporogenous structure* phialides mostly borne singly on the vegetative hyphae or sometimes in groups of two or three on short, poorly differentiated conidiophores. *B-sporogenous structure* conidiophores with the finely echinulate stalk, pale brown, erect, simple or irregularly branched, phialides borne singly or in groups of two or three on the near apex or short branch. *Phialides* ovoid, ellipsoidal, cylindrical or lycethiform in basal portion, tapering often abruptly into a long distinct neck, 5-22.3 (-25.3) × (1.5-) 1.9-5.5 (-6.2) µm, the few having proliferation. *Conidia* one-celled, hyaline, smooth-walled,

obovoid, ellipsoidal, long ellipsoidal to fusiform, (3.5-) 4.5-10.4 (-13) × (-2) 3.5-5 (-7) μm, forming divergent, dry long chains.

Key to the species and some thermotolerant relatives of *Paecilomyces*

1. Sporogenous structure monophialidic and definite conidiophores..... ***P. biformis***
1. Sporogenous structure mainly monophialidic or definite conidiophores 2

2. Sporogenous structure mainly monophialidic 3
2. Sporogenous structure definite conidiophores 4

3. Conidia small, 3-4 × 2-3 μm ..... ***P. inflatus***
3. Conidia large, 5.2-14 × 2.5-5.1 μm..... ***P. major***

4. Conidia mostly subglobose or ellipsoidal..... 5
4. Conidia mostly cylindrical, ellipsoidal or broadly fusiform..... 6

5.Conidia globose, small, 2.5-4 × 1.5-3 μm.....***P. zollerniae***
5. Conidia subglobose, large, 3.5-7 × 2.2-4 μm ..... ***P. niveus***

6. Conidia mostly ellipsoidal or broadly fusiform..... 7
6. Conidia mostly cylindrical or ellipsoidal 8

7. Conidia ellipsoidal, spindly or clavate, 7-11.5 × 5.7-4.3 μm ..... ***P. clavisporus***
7. Conidia ellipsoidal or fusiform, 6-8.5 ×2.5-3.5 μm .....***P. aerugineus***

8. Colony green, conidia less than 2 μm wide.....***P. leycettanus***
8. Colony in other colors, conidia more than 2 μm wide 9

9. Colony wine red, colonies in variable, 4.5-11 × 2-9.5 μm.....***P. aegyptiacus***
9. Colony in other colors, conidia mostly cylindrical..... 10

10. Colony in white or other bright colors 11
10. Colony in other colors..... 12

11. Colony orange, conidia cylindrical to ellipsoidal, 6-10 × 3-6 μm.....***P. crustaceus***
11. Colony white to yellow, conidia cylindrical to subglobose, 4-8 × 2-5.5 μm***...P. taitungiacus***

12. Colony white to bright brown, conidia cylindrical, 4-8.7 × 1.5-5 μm.....***P.fulvus***
12. Colony yellow to yellow-green, conidia long cylindrical, 9-13 × 2.5-4 μm ..... ***P.verrucosus***

Production of laccase

The qualitative experiment results showed that the fungus *P. biformis* GZDXIFR-H28 exhibited laccase activities on the PDA plates containing different concentrations of O-methoxyphenol. The lower concentration, 0.02%-0.03% O-methoxyphenol is good for colony growth, whereas the stronger

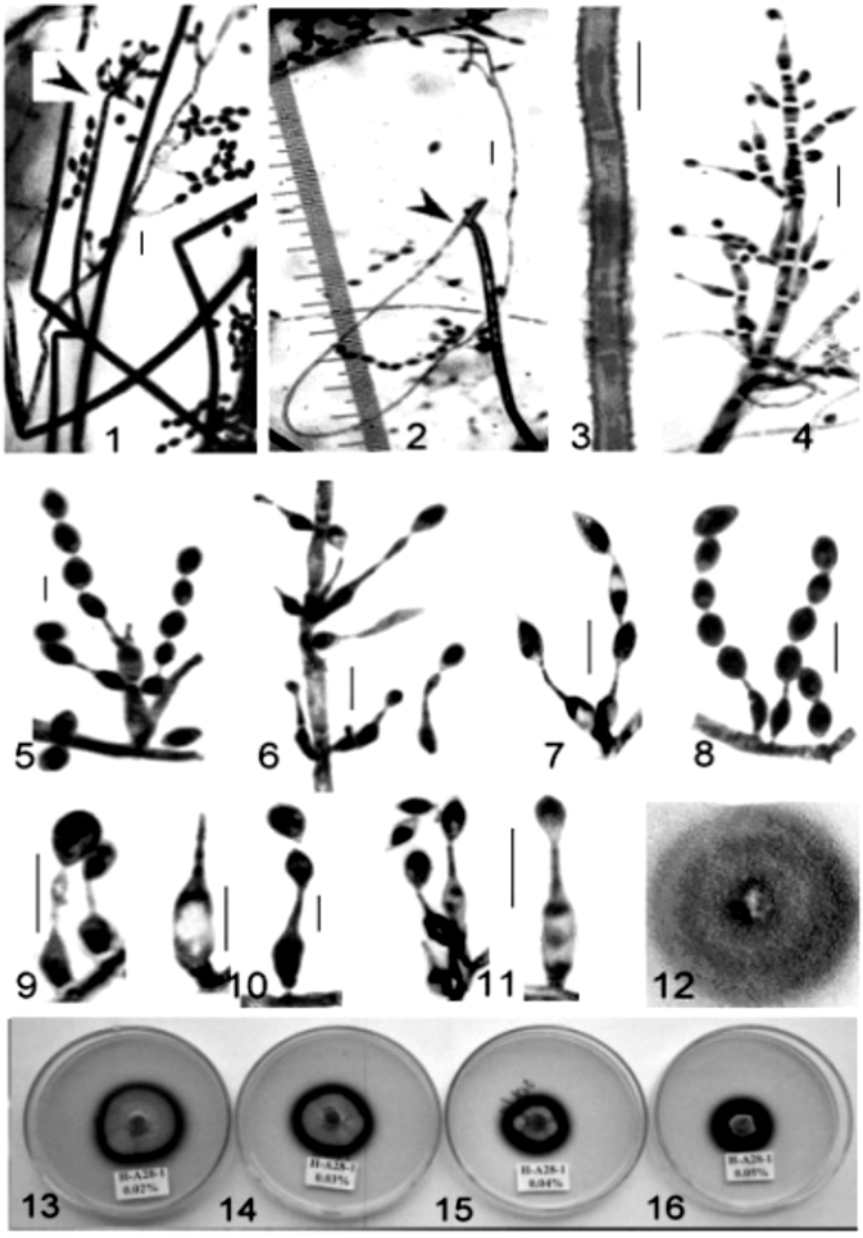

Fig. 1-16. The sporogenous structures of *Paecilomyces biformis*. **1, 2.** Vegetative hyphae − conidiophore connection (arrowed). **3.** Magnified echinulate stalk of conidiophore. **4.** Upper part of conidiophores with phialides. **5.** Short conidiophore. **6-8.** Phialides borne directly on the vegetative hyphae. **9-11.** A few of magnified phialides. **12.** A colony. **13-16.** Catalysis effect of laccase from *P. biformis* on PDA containing O-methoxyphenol (13. 0.02% O-methoxyphenol, **14.** 0.03% O-methoxyphenol ,15. 0.04% O-methoxyphenol. **16.** 0.05% O-methoxyphenol; 4 days). Bars = 10 µm

enzyme activities exhibit on the medium containing 0.05% O-methoxyphenol based on detecting the colony diameter and size of oxidation zone (Figs 13-16).

Discussion

The anamorphic pleomorphism in fungi could be usually divided into three kinds: (1) pleomorphism with conidia of the same ontogenetic type, (2) pleomorphism with conidia of different type, and (3) pleomorphism of conidiomata (Carmichael, 1981). *Paecilomyces biformis* has two-type of sporogenous structure consisting of solitary phialides and simple conidiophores. This character could be as a new example of anamorphic pleomorphism in the kind of (3), pleomorphism of conidiomata.

The monophialidic character of *P. biformis* was related to some species of *Acremonium*. However, the phialides with ampullaceously inflated basal portion and tapering into a distinct neck reveal this species belonging to the genus *Paecilomyces*. Now the monophialidic species that belong to the genus *Paecilomyces* have *P. inflatus* (Gams, 1971; Samson, 1974), *P. ampullaris* (Matsushima, 1971), *P. ampulliphoris* (1975), *P. iriomoteanus* (Matsushima, 1975), *P. major* (Chu *et al.*, 2004; Liang *et al.*, 2006a), *P. furcatus, P. cinereus* (Liang *et al.*, 2006b) and *P. curticatenatus* (Han *et al.*, 2007). *Paecilomyces biformis* can be distinguished from other monophialidic *Paecilomyces* species by its biform of sporogeous structure, finely echinulate conidiophores and large conidia (4.5-10.4 × 3.5-5 μm). Recently (Liang *et al.*, 2006b), we analysed a set of sequence data generated from ribosomal DNA (ITS). The analysis result of phylogenetic tree supported the establishment of *P. biformis* as a new taxon (Liang *et al*., 2006b).

Samson (1974) doubted that *P. ampullaris* should be in the genus *Paecilomyces* because specimen studied showed that it has a sympodial elongation. When we studied Matushima's original description and line drawings (Matushima, 1971), it was discovered that conidial aggregation of this species was a typical basipetal long chain not sympodial conidiation. So we think that the fungus *P. ampullaris* should instead be placed into the genus *Paecilomyces*. Although *P. biformis* GZDXIFR-H28 resembles *P. ampullaris* in bearing single phialides and the shape of conidia, this fungus can be differentiated by echinulate conidiophores, large conidia (4.5-10.4 × 3.5-5 μm) and thermotolerant features from *P. ampullaris*.

Paecilomyces crustaceus (Apinis & Chesters) Yaguchi, Someya & Udagawa is very similar to *P. biformis* GZDXIFR-H28 in shape and size of phialides and conidia, and a higher optimum temperature at about 40ºC (Samson, 1974). *Paecilomyces crustaceus* however has no finely echinulate conidiophores or phialides borne singly.

Species of *Paecilomyces* from China have been discussed by Liang *et al*. (2005). Most members of the genus *Paecilomyces*, have optimal growth temperatures ranging between 35ºC and 40ºC. *P. leycettanus* (H.C. Evans & Stolk) Stolk, Samson & H.C. Evans, *P. byssochlamydoides* Stolk & Samson, *P. crustaceus* and some strains of *P. variotii* Bainier can grow above 40ºC (Samson, 1974). Subsequently a few thermotolerant species in the genus, *P. verrucosus* Samson & Tansey (Samson & Tansey, 1975), *P. aegyptiacus* S. Ueda & Udagawa (Ueda and Udagawa, 1983), *P. taitungiacus* K.Y. Chen & Z.C. Chen (Chen and Chen, 1996) and *P. major* (Chu *et al.*, 2004) have been reported. So far there are 13 thermotolerant taxa in the genus *Paecilomyces*. It is interesting that most of thermotolerant species of *Paecilomyces* in China as we investigated are monophialidic species. These events imply perhaps that the monophialidic series in *Paecilomyces* proposed by Onions and Barron (1967) really exist in nature.

More than 40 genera of microorganisms have been reported to produce laccase. Traditionally the important laccase-producing fungi are mainly white-rot basidiomycete fungi (Pointing, 2001), such as *Phanerochaete chrysosporium* Burds., *Coriolus hirsutus* (Wulfen) Quél., *Trametes versicolor* (L.) Lloyd, *Phlebia radiata* Fr., *Pleurotus pulmonarius* (Fr.) Quel. and *Pycnoporus cinnabarinus* (Jacq.) P. Karst. Besides the mitosporic fungi *Aspergillus* and *Sporotrichum,* two strains *P. inflatus* from an urban compost which produced laccase, were reported (Kluczek-Turpeinen *et al.*, 2003). The results of our experiments showed that laccase production of *P. biformis* GZDXIFR-H28 has comparability with those strains of *P. inflatus*.

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