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Research Note

# A New Record of *Penicillium pimiteouiense* from Beach Soil in Malaysia

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**Abstract** Three isolates of *Penicillium pimiteouiense* were recovered from sandy beach soil samples in Penang Island, Peninsular Malaysia. All the isolates were identified based on morphological characteristics and phylogenetic analysis of internal transcribed spacer regions and β-tubulin gene. This is a first record of *P. pimiteouiense* in Malaysia.

**Keywords** Beach soil, β-tubulin, ITS regions, Morphology, *Penicillium pimiteouiense*

*Penicillium* species are abundant in various soil environments such as cultivated soil, forest soil, desert soil and also beach soil [1]. Traditionally, identification of *Penicillium* species was based on morphological characteristics [2]. Nowadays, molecular methods have been used extensively for studying phylogenetic relationships among closely related *Penicillium* species [3, 4]. During the studies on biodiversity of fungal community and the occurrence of the genus *Penicillium* in sandy beach soil in Penang Island, Peninsular Malaysia, a species of *Penicillium* previously unreported in Malaysia was encountered. Based on morphological and molecular characteristics, this species was identified as *P. pimiteouiense*.

Soil samples were collected from a sandy beach in Penang Island, Peninsular Malaysia in August 2010. Each soil sample was taken from approximately 10~15 cm depth, air dried and stored in paper bags at 4°C until used. *Penicillium* isolates were obtained from direct isolation technique [5]. The soil was weighted 0.3 g and evenly distributed on malt extract agar (MEA). Three replicates were prepared for each soil sample. The plates were incubated for 5 to 7 days at 25°C until the growth of fungal colonies was observed.

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Individual colonies were picked with a sterile inoculation needle and transferred onto new MEA media. Three cultures were examined, A4S2-19, A4S2-20, and A4S2-21 and are deposited in Plant Pathology Lab, School of Biological Sciences, Universiti Sains Malaysia.

Morphological features were observed on Czapek yeast extract agar (CYA; Himedia, Mumbai, India), MEA (Himedia), and yeast extract sucrose (YES) agar. Three-point inoculation were done in 9 cm Petri dishes plates using a dense conidial suspension and incubated in the dark at 25°C for 7 days. Conidial morphology on MEA media was measured and compared with description by Peterson *et al.* [6]. Morphological features of the isolates are shown in Table 1 and Fig. 1.

Morphological characteristics of the isolates agreed with the description by Peterson *et al.* [6]. *Penicillium pimiteouiense* isolates were identified based on the production of yellow to red exudates on the upper surface of the colonies on MEA, CYA and YES as well as the yellow to orange colour on the lower surface (Fig. 1). Colony diameters of *P. pimiteouiense* isolates were 20~24 mm on CYA, 21~30 mm on MEA and 25~31 mm on YES. No reaction was observed for Ehrlich test. Colonies on CYA was radially sulcate, yellow exudates on the upper surface and yellow to orange colour on the lower surface (Fig. 1A and 1D). On MEA, colonies were thick, cottony and radially sulcate with white mycelium, conidial areas were light green and yellow to red exudates were present (Fig. 1B and 1E). The colonies on YES were wrinkled, white and the lower surface was orange to brown (Fig. 1C and 1F).

Conidiophores were monoverticillate and nonvesiculate on MEA, arising from aerial hyphae (Fig. 1G). Stipes were simple, smooth-walled and short (12~20 × 1.9~2.1 μm). Phialides were ampulliform (4.7~5.8 × 1.4~3.2 μm). Conidia were born in short chains, globose to subglobose, finely roughened with 2.0~3.2 μm in diameter (Fig. 1H and 1I).

To confirm the morphological results, molecular

**Table 1.** Comparison of cultural and morphological characteristics between the present isolates and *Penicillium pimiteouiense* described previously

| Colony | Present isolate | *P. pimiteouiense*[a] |
|---|---|---|
| CYA | White with 10~14 sulcate, yellow exudates produced, yellow soluble pigments produced, reverse yellow orange | White with 4~5 sulcate, yellow exudate, brown soluble pigment produced, reverse dark brown |
| MEA | White with 5~7 sulcate, red exudates produced, yellow soluble pigments produced, reverse yellow orange | White with mass light olive gray conidial colour |
| YES | White with many irregularly radial sulcate, exudates absent, yellow soluble pigments produced, reverse yellow orange | N/A |
| Size (in diameter) | 20~24 mm on CYA<br>21~30 mm on MEA<br>25~31 mm on YES | 16~18 mm on CYA<br>20~22 mm on MEA |
| Stipe | Simple, smooth-walled, short, 12~20 × 1.9~2.1 μm | Short, 5~10 (~16) × 2~3 μm |
| Phialide | Ampulliform, 4.7~5.8 × 1.4~3.2 μm | Ampulliform, 5~6 (~8) × 1.5~2.2 (~3) μm |
| Conidia | Born in short chains, globose to subglobose, finely roughened, 2.0~3.2 μm in diameter | Born in short chains, globose to subglobose, finely roughened, 1.5~3 μm in diameter |

CYA, Czapek yeast extract agar; MEA, malt extract agar; YES, yeast extract sucrose agar; N/A, not available in the previous description.
[a]Sources of description [6].

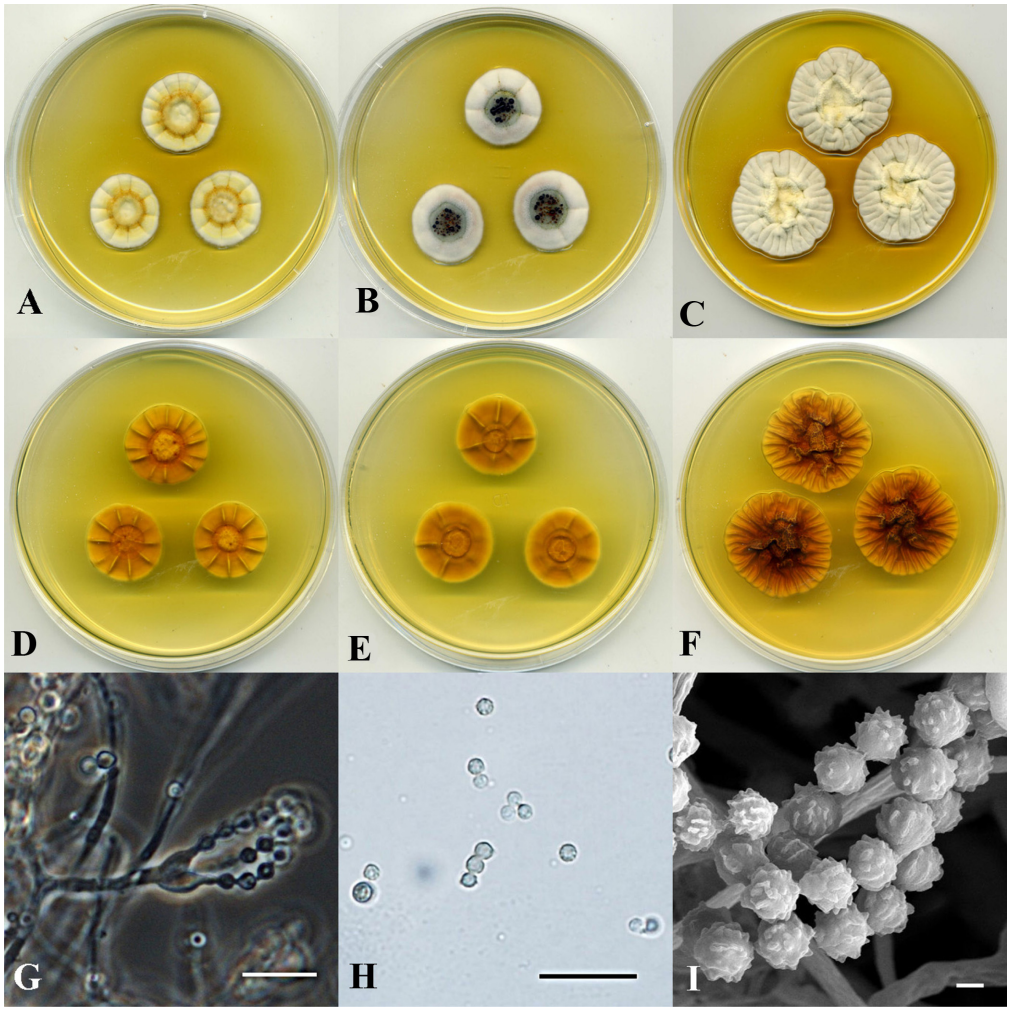


**Fig. 1.** *Penicillium pimiteouiense* colonies grown on Czapek yeast extract agar (A, D), malt extract agar (B, E), and yeast extract sucrose agar (C, F) for 7 days at 25°C, conidiophores (G), conidia (H, I) (scale bars: G, H = 10 μm, I = 1 μm).

identification of the isolates were carried out. Mycelia for DNA extraction were grown in potato dextrose broth using Universal bottles and incubated at 25°C. Mycelia were harvested by filtration when mycelium was visible with no sporulation, generally after 16~48 hr. Mycelia were frozen and lyophilized, and then crushed using liquid nitrogen. Genomic DNA was extracted using Invisorb Spin Plant Mini Kit (STRATEC Molecular GmbH, Berlin, Germany) according to the manufacturer's protocol. For amplification of internal transcribed spacer (ITS) regions, ITS1 and ITS4 primers were used [7], while β-tubulin gene was amplified using Bt2a and Bt2b primers [8].

The DNA sequences were analysed for phylogenetic relationship using Molecular Evolutionary Genetic Analysis (MEGA5) software [9]. The sequences of *P. pimiteouiense* isolates were compared with sequences in the GenBank by using Basic Local Alignment Search Tool (BLAST). The combined datasets of both ITS regions and β-tubulin were used to generate phylogenetic tree. Maximum likelihood (ML) tree was constructed by using Kimura 2-parameter substitution model [10]. Tree was inferred using the ML heuristics search option with nearest-neighbour-interchange. Bootstrap analysis was performed with 1,000 replications in order to determine the support for each clade. ITS regions and β-tubulin gene sequences (accession No. as indicated in Table 2) of the three isolates were identical to the type strain CBS 102479 of *P. pimiteouiense* [6]. In addition, the phylogenetic tree showed that all three isolates were grouped in a distinct clade together with type strain CBS 102479 of *P. pimiteouiense* with 100% bootstrap value support (Fig. 2). The results confirmed that the three isolates were *P. pimiteouiense*.

Although members of the genus *Penicillium* are common saprophytes in the beach soil environment, they may act as opportunistic pathogens, especially to immunocompromised patients [11]. Moreover, *P. pimiteouiense* was first isolated from polycystic kidney cell cultures by Peterson *et al.* [6] which suggested that the species may play a role in polycystic kidney disease [11]. *Penicillium pimiteouiense* has also been isolated from chicken litter samples by Wadud *et al.* [12] and from agricultural field in India [13]. However, this study is the first recorded occurrence of *P. pimiteouiense* in Malaysia.

**Table 2.** Lists of GenBank accession number

| Isolates | Species | GenBank accession No. | |
|---|---|---|---|
| | | ITS regions | β-Tubulin |
| A4S2-19 | *Penicillium pimiteouiense* | KC344972 | KC344994 |
| A4S2-20 | *P. pimiteouiense* | KC344973 | KC344995 |
| A4S2-21 | *P. pimiteouiense* | KC344974 | KC344996 |

ITS, internal transcribed spacer.

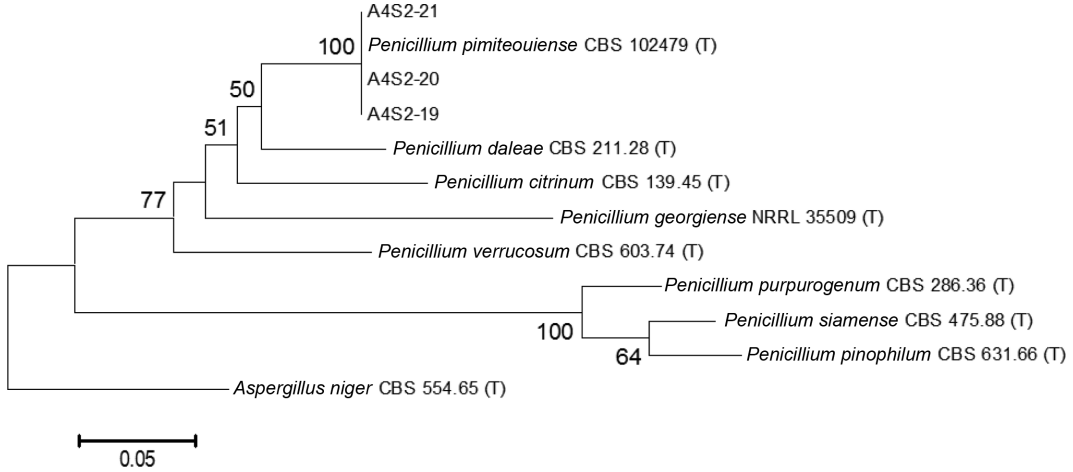


**Fig. 2.** Maximum likelihood tree for *Penicillium pimiteouiense* based on a combined internal transcribed spacer regions and β-tubulin sequences. Bootstrap values (> 50%) are showed at the nodes. The bar indicates the number of substituitions per site. The mark (T) indicates type strain.

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