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Research Note

The First Report of *Penicillium georgiense* in Malaysia

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Abstract *Penicillium georgiense* was isolated from sandy beach soil from Batu Ferringhi beach, Penang Island, Malaysia. The identification was based on morphological characteristics and phylogenetic analysis of internal transcribed spacer regions and β-tubulin sequences. To the best of our knowledge, this is the first report of *P. georgiense* in Malaysia.

Keywords Beach soil, β-Tubulin, ITS regions, Morphology, *Penicillium georgiense*

Penicillium georgiense was first isolated from peanut-field soils in Georgia, USA, and was reported to show specificity for conidial heads of *Aspergillus* section *Nigri* [1, 2]. Previously, the only published data on the occurrence of *P. georgiense* in soil was the original report by Peterson and Horn [1]. During a biodiversity survey of microfungi in sandy beach soil, *P. georgiense* was isolated and the species identified based on morphological characteristics as well as internal transcribed spacer (ITS) regions and β-tubulin gene sequences. Therefore, this is the first report of the occurrence of this species in sandy beach soil in Malaysia.

Eight *P. georgiense* isolates were recovered from sandy beach soil in Batu Ferringhi (N 5°28', E 100°15'), Penang Island, Malaysia. Isolation was performed on malt extract agar (MEA; Himedia, Mumbai, India). Soil samples were taken from approximately 10~15 cm depth, air dried, and stored in paper bags at 4°C until use. Soil was weighed and 0.3 g was evenly distributed on three plates of MEA. The plates were incubated for five to seven days at 25°C until the growth of fungal colonies was observed. Individual colonies were picked with a sterile inoculation needle and transferred onto new MEA plates. *P. georgiense* isolates were obtained using the direct isolation technique [3]. All eight isolates were deposited in the culture collection Plant Pathology Lab, School of Biological Sciences, Universiti Sains Malaysia.

The macromorphology of all the isolates was observed on Czapek yeast extract agar (CYA; Himedia), MEA, and yeast extract sucrose agar (YES). Three-point inoculation was carried out in 9 cm petri dishes using a dense conidial suspension, followed by incubation in the dark at 25°C for 7 days. The micromorphology was studied on MEA and compared with the description of Peterson and Horn [1]. For microscopic observation, lactic acid (60%) without color dye and lactophenol blue were used as the mounting medium. Twenty-five conidia were randomly measured from each isolate. Morphological features of the isolates are shown in Table 1 and Figs. 1 and 2.

Generally, morphological characteristics of the *P. georgiense* isolates were in agreement with the description by Peterson and Horn [1] although some variations were observed between isolate A3S1-14 and the other seven *P. georgiense* isolates (Table 1). For the seven isolates of *P. georgiense*, the colonies on CYA were 13~19 mm in diameter after seven days, and consisted of velvety, white mycelium with olive green conidial masses, and an absence of exudates (Fig. 1A and 1D). On MEA, the colonies were 18~25 mm in diameter after seven days, velvety, and consisted of dark green conidial masses with white mycelium in the center, and an absence of exudates (Fig. 1B and 1E). On YES, the colonies were 13~17 mm diameter after seven days, and consisted of velvety, dark green conidial masses with white mycelium in the center, and an absence of exudates (Fig. 1C and 1F).

The conidiophores of the seven isolates of *P. georgiense* were biverticillate, with smooth-walled stipes, bearing short conidial chains (Fig. 1G and 1H). Metulae measured 8.5~11 × 2~3.5 µm, whilst phialides were cylindrical and measured

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Table 1. Comparison of cultural and morphological characteristics between the isolates of the present study and *Penicillium georgiense* isolates described by Petersen and Horn [1]

	Isolates (A2S2-D35, A2S4-D60, A3S1-37, A3S2-6, A3S2-20, A3S3-31, A3S6-47)	Isolate A3S1-14	*P. georgiense*[a]
CYA	White with olive green conidial masses, velvety, exudates absent	Dark green conidial masses with white mycelium at the margin, radially sulcate, velvety, yellow soluble pigments produced, exudates absent	Celandine green, velvety, moderate exudate present in some isolates
MEA	Dark green conidial masses with white mycelium in the center, velvety, exudates absent	Dark green conidial masses with white mycelium at the margin, radially sulcate, velvety, yellow soluble pigments produced, exudates absent	Near celandine green with mycelium in the central area, velvety, exudates absent
YES	Dark green conidial masses with white mycelium in the center, velvety, exudates absent	Dark green conidial masses with white mycelium at the margin, wrinkled, velvety, yellow soluble pigments produced, exudates absent	N/A
Diameter	13~19 mm on CYA 18~25 mm on MEA 13~17 mm on YES	21~29 mm on CYA 26~29 mm on MEA 27~34 mm on YES	15~21 mm on CYA 21~23 mm on MEA
Conidiophore	Biverticillate, bearing short conidial chains, smooth-walled	Biverticillate, bearing short conidial chains, smooth-walled	Biverticillate, bearing short conidial chains, smooth-walled.
Metulae	8.5~11 × 2~3.5 μm	12.5~18 × 2.5~3 μm	(12)~15~(17.5) × 2.5~3 μm
Phialid	9~11 × 2~3 μm	6~10 × 2.5~3 μm	8~10 × 2.5~3 μm
Conidia	Born in short chains, globose to subglobose, smooth-walled, 2.5~3.5 × 2~3 μm	Born in short chains, globose to subglobose, smooth-walled, 3~3.5 × 2~3 μm	Born in short chains, ellipsoidal to subglobose, smooth-walled, 3~4 (4.5) × 2.5~3 μm

N/A indicates not available in the previous description [1].
CYA, Czapek yeast extract agar; MEA, malt extract agar; YES, yeast extract sucrose agar; N/A, not available.
[a]Source of description [1].

9~11 × 2~3 μm. Conidia observed were smooth, globose to subglobose, and were 2.5~3.5 × 2~3 μm in diameter (Fig. 1I).

When compared with the other seven isolates, isolate A3S1-14 exhibited a larger colony diameter on all three culture media, along with production of yellow soluble pigments (Table 1). Isolate A3S1-14 colonies on CYA were 21~29 mm in diameter after seven days, radially sulcate, and consisted of velvety, dark green conidial masses with white mycelium at the margin. Yellow soluble pigments were produced but exudates were absent (Fig. 2A and 2D). On MEA, the colonies were 26~29 mm in diameter after seven days, radially sulcate, and consisted of velvety, dark green conidial masses with white mycelium at the margin, with yellow soluble pigments present, but exudates absent (Fig. 2B and 2E). On YES, colonies were 27~34 mm in diameter after seven days, wrinkled, velvety, with dark green conidial masses, white mycelium at the margin with yellow soluble pigments, and absence of exudates (Fig. 2C and 2F). The conidial size of isolate A3S1-14 was also larger than the seven other isolates, but the microscopic characteristics of all eight isolates were very similar (Table 1). A certain degree of morphological variability might indicate intraspecific genetic variation, similar to that reported in *Penicillium raistrickii* [4].

To confirm the morphological results, molecular identification was carried out. Cultures were grown for DNA extraction in potato dextrose broth using universal bottles and incubated at 25°C. The mycelium was harvested by filtration after 16~48 hr, frozen and lyophilized, and then crushed using liquid nitrogen. Genomic DNA was extracted using Invisorb Spin Plant Mini Kit (STRATEC Molecular GmbH) according to the manufacturer's instructions. For the amplification of ITS regions, the ITS1 and ITS4 primers were used [5], while β-tubulin was amplified using Bt2a and Bt2b primers [6]. PCR conditions and PCR cycles for amplification were adapted from White *et al.* [5] for ITS, and from Glass and Donaldson [6] for β-tubulin, with some modifications. The annealing temperature used for both ITS and β-tubulin was 58°C.

The ITS regions and β-tubulin gene sequences of all eight *P. georgiense* isolates were deposited in GenBank with accession Nos. KC329462~KC329469 for ITS, and KC344981~KC344988 for β-tubulin. The sequences of *P. georgiense* isolates were compared with sequences in GenBank by using the Basic Local Alignment Search Tool (BLAST). The results of the BLAST search showed 98% similarity for ITS regions and 95~96% similarity for β-tubulin with sequences of *P. georgiense* isolates examined by Peterson and Horn [1].

Phylogenetic relationships of the DNA sequences were

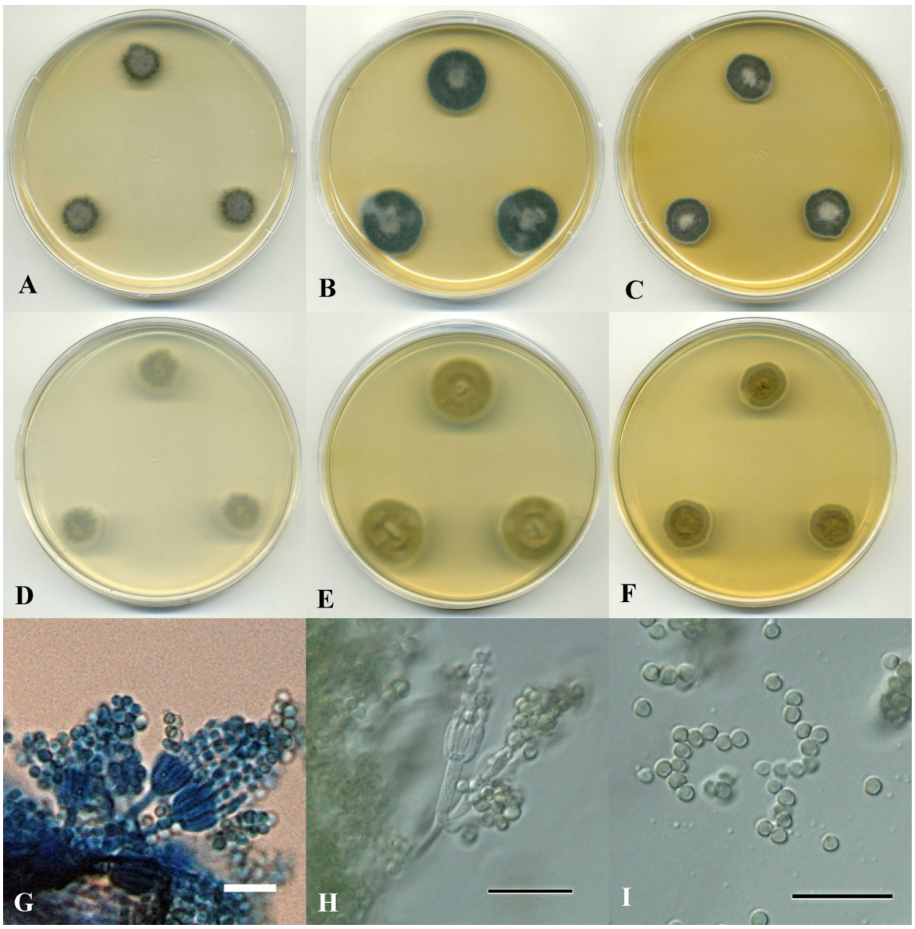

Fig. 1. Morphological characteristics of seven isolates of *Penicillium georgiense* (A2S2-D35, A2S4-D60, A3S1-37, A3S2-6, A3S2-20, A3S3-31, and A3S6-47). Colonies grown on Czapek yeast extract agar (A, D), malt extract agar (B, E), and yeast extract sucrose agar (C, F) after seven days at 25°C. Conidiophores (G, H) and conidia (I) (scale bars: G~I = 10 μm).

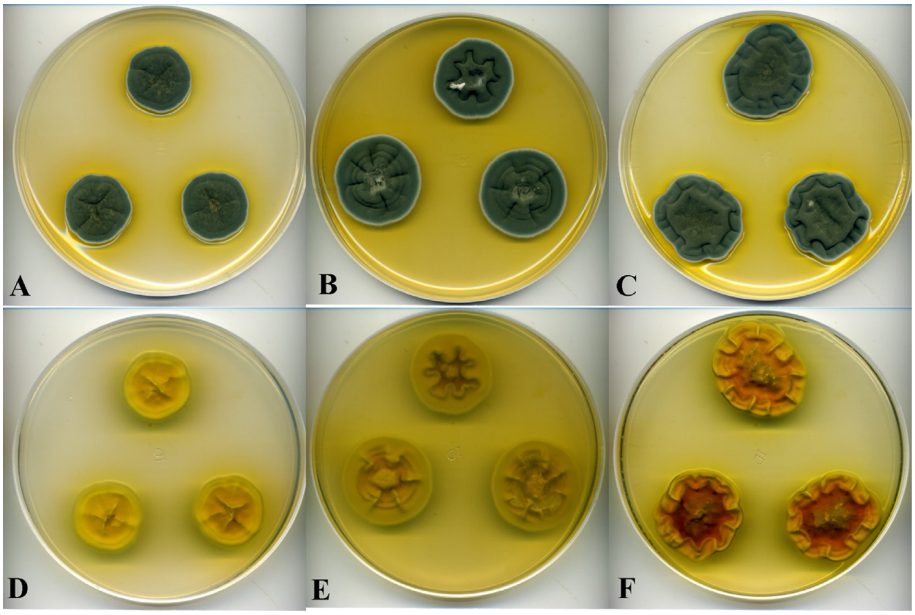

Fig. 2. Morphological characteristics of *Penicillium georgiense* isolate A3S1-14. Colonies grown on Czapek yeast extract agar (A, D), malt extract agar (B, E), and yeast extract sucrose agar (C, F) after seven days at 25°C.

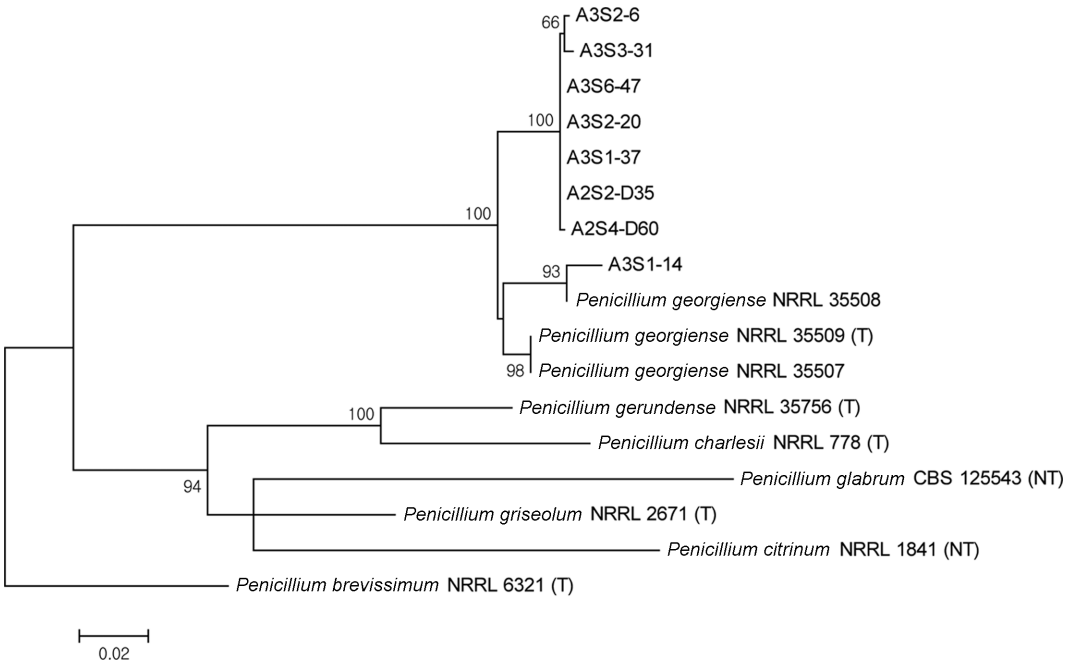

Fig. 3. Maximum likelihood tree of *Penicillium georgiense* isolates based on analysis of combined internal transcribed spacer and β-tubulin sequences. Bootstrap values (> 50%) are shown at the nodes. The bar indicates the number of substitutions per site.

analyzed using Molecular Evolutionary Genetic Analysis (MEGA5) software [7]. The combined dataset of both ITS and β-tubulin were analyzed using the maximum likelihood (ML) method, constructed using the Kimura two-parameter substitution model [8]. The tree was inferred using the ML heuristics search option with nearest-neighbor-interchange. Bootstrap analysis was performed with 1,000 replications in order to determine the support for each clade. *Penicillium georgiense* is in section *Aspergillioides*, and therefore other species from the same section, as well as from different sections, were included in the phylogenetic analysis. Five *Penicillium* species included in the analysis were *P. glabrum* (section *Aspergillioides*), *P. citrinum* (section *Citrina*), *P. gerundense* (section *Charlesia*), and *P. charlesii* (section *Charlesia*) from subgenus *Aspergillioides*, and an outgroup (*P. brevissimum*) from section *Ramigena.*

The phylogenetic analysis resulted in three highly supported subclades. Seven isolates were grouped together in the same clade and one isolate (A3S1-14) was grouped with the *P. georgiense* type strain NRRL 35508 (Fig. 3). The other two types of strain (NRRL 35507 and NRRL 35509) formed separate subclades. The results indicated that the *P. georgiense* isolates from beach soil might represent different phylogenetic strains of *P. georgiense*, which may be either different phylogenetic species or genetically differing intraspecific lineages. The results of the present study are similar to a study by Scott *et al.* [9] on *P. chysogenum*, in which phylogenetic analysis identified three lineages, representing distinct phylogenetic species. Although the isolates of *P. georgiense* in the present study might be different phylogenetic strains from the strain reported by Petersen and Horn [1, 2], this study, to the best of our knowledge, is the first recorded occurrence of *P. georgiense* in Malaysia.

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