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Occurrence and identification of *Penicillium* and *Talaromyces* species from beach sand

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ABSTRACT

Aims: *Penicillium* and *Talaromyces* were among the species of microfungi that inhabit beach sand in Batu Ferringhi Beach, Penang Island, Malaysia. Previously, *Talaromyces* was described as the sexual stage of *Penicillium*, but both are now accepted as separate genera based on molecular phylogeny. The aim of the present study was to identify species of *Penicillium* and *Talaromyces* that are present in beach sand in Malaysia.
Methodology and results: Species identities were confirmed according to similarities of the internal transcribed spacer regions and β-tubulin gene sequences and a phylogenetic analysis based on both regions/gene. Nine *Penicillium* spp. were identified as *P. georgiense*, *P. chermesinum*, *P. pimiteouiense*, *P. citrinum*, *P. oxalicum*, *P. daleae*, *P. rolfsii* and *Penicillium* sp. and the four *Talaromyces* spp. were *T. siamense*, *T. atroroseus*, *T. minioluteus* and *T. fusiformis*.
Conclusion, significance and impact of study: These findings showed that beach sand harboured a variety of *Penicillium* and *Talaromyces* species. The occurrence of *Penicillium* and *Talaromyces* in beach sands is associated with the organic matter in the sand, which provides suitable substrates and nutrient sources. Due to this, beach sand might harbour many potentially pathogenic or opportunistic species that may pose a health concern to immunocompromised individuals.

Keywords: β-tubulin, beach sand, internal transcribed spacer regions, *Penicillium*, *Talaromyces*

INTRODUCTION

Microfungi inhabit many soil types and include saprophytes, mutualists and pathogens of plants, animals and humans (Bills *et al.*, 2004). Microfungi in soil ecosystems mainly function as decomposers of organic matter; however, some species are opportunistic pathogens. Microfungi are often encountered in beach sand (Latiffah *et al*., 2011; Whitman *et al.*, 2014) and their survival and dispersal depend on the characteristics of the beach, season, tides, animals and human activities (Mendes *et al*., 1998).

Penicillium and *Talaromyces* are well-known filamentous microfungi that play important roles in natural ecosystems, particularly as decomposers. Many species of *Penicillium* and *Talaromyces* are distributed worldwide and occupy a wide range of ecological habitats, including soils, indoor environments, plants and animals. *Penicillium* has been isolated from beach sand in several parts of the world, such as on Spanish Mediterranean beaches (Larrondo and Calvo, 1989), the Attica area in Greece (Papadakis *et al*., 1997), the Ligurian coast of Italy (Salvo and Fabiano, 2007), Fethiye Beach, Turkey (Güçlü *et al.*, 2010) and the sandy beaches along the Algerian western coast (Matallah-Boutiba *et al.*, 2011). *Penicillium* is one of the most frequently isolated genera in the sand of the Casa Caiada and Bairro Novo beaches, in Brazil (Gomes *et al.*, 2008). In these studies, the species identity was not determined. *Talaromyces* species may have also been present among the isolated *Penicillium* species, as this genus had not been recognised as a distinct genus from *Penicillium* at the time of these studies.

Talaromyces was originally introduced to group the sexual stage of *Penicillium* (Benjamin, 1955). Molecular phylogenetic analyses using internal transcribed spacer region (ITS), small subunit and/or large subunit of rRNA, and RNA polymerase II largest subunit showed *Talaromyces* and species of *Penicillium* subgenus *Biverticillium* formed a monophyletic clade (Houbraken and Samson, 2011; Samson *et al.*, 2011), which indicated these two genera are distinct. Moreover, several studies have suggested that subgenus *Biverticillium* results in *Penicillium* being polyphyletic (LoBuglio *et al*., 1993; Peterson, 2000; Heredia *et al.*, 2001; Wang and Zhuang, 2007). Thus, *Penicillium* subgenus *Biverticillium* was combined with *Talaromyces* according to the one fungus, one name concept or single nomenclature of fungi

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(Houbraken and Samson, 2011; Samson *et al.*, 2011). Currently, *Penicillium* and *Talaromyces* are accepted as distinct genera.

Penicillium and *Talaromyces* are widely distributed worldwide. In addition to decomposers that maintain ecosystem functioning, many species within these genera are important plant pathogens, mycotoxin producers contaminating food products and antibiotics producers (Yilmaz *et al.*, 2014).

The tropical beaches in Malaysia provide a potentially suitable habitat for a wide diversity of *Penicillium* and *Talaromyces* species. During a diversity survey of the microfungi in beach sand at Batu Ferringhi Beach, Penang Island, Malaysia, *Penicillium* isolates with various conidiophore branching patterns were recovered, which might include *Talaromyces* isolates. Despite being frequently recovered from soils, especially in tropical areas (Cruz *et al.*, 2013; Barbosa *et al*., 2018), there is little information on the occurrence of *Penicillium* and *Talaromyces* in beach sand in Malaysia. Therefore, the objective of this study was to molecularly identify *Penicillium* and *Talaromyces* species in beach sand from Batu Ferringhi Beach, which can address the gap in our knowledge of the presence of *Penicillium* and *Talaromyces* species in Malaysian beach sand.

MATERIALS AND METHODS

Beach sand samples were collected from beach areas of Batu Ferringhi, Penang. There were four sampling areas (the beach area from Tropical Spice Garden, Bayview Hotel, Golden Sand Hotel and Rasa Sayang Resort) and six samples were collected from each site. The distance between each sampling site in an area was 20 m.

In total, 146 *Penicillium* isolates were obtained from 19 beach sand samples from Batu Ferringhi Beach, Penang Island, Malaysia. All the isolates were morphologically identified to sort them into morphospecies or morphological groups. Morphological identification was based on the procedures described by Samson *et al.* (2010), Samson *et al.* (2011) and Houbraken and Samson (2011). The isolates were inoculated at three points positions on Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA), Yeast Extract Sucrose Agar (YES) and Creatine Sucrose Agar (CREA). The plates were incubated at 27 ± 1 ºC for 7 days. The colony characteristics observed were colony appearance on the upper and lower surfaces of MEA, CYA and YES; colony diameter; and acid production on CREA represented by changes in colour from purple to yellow. The main microscopic characteristics observed were conidiophore branching patterns and conidial shapes.

As the microscopic and macroscopic characteristics of each morphospecies were very similar, 45 isolates were chosen as representatives of each morphospecies for molecular characterisation. Mycelia for DNA extraction were grown in universal bottles with potato dextrose broth at 25°C and harvested by filtration after 16-48 h. Mycelia were frozen, lyophilised and then crushed in liquid nitrogen. Genomic DNA was extracted using an Invisorb Spin Plant Mini Kit (Stratec Molecular GmbH, Berlin, Germany) according to the manufacturer's protocol. For amplification of the ITS regions, ITS1 and ITS4 primers were used (White *et al.*, 1990) and the gene encoding the β-tubulin gene was amplified using the primers Bt2a and Bt2b (Glass and Donaldson, 1995).

Polymerase Chain Reactions (PCR) for ITS regions and the β-tubulin gene were performed in a Peltier Thermal Cycler Model PTC-100 (MJ-Research, Watertown, MA, USA). The DNA amplifications were performed in a total volume of 25 µL containing 0.5 µL of genomic DNA, 4.0 mM of magnesium chloride, 0.8 mM deoxyribonucleotide triphosphates and 0.625 U of *Taq* polymerase (Promega, Madison, WI, USA). The primer concentrations to amplify ITS and β-tubulin gene sequences were 0.5 µM and 0.2 µM, respectively. The PCR cycles started with an initial denaturation at 95 ºC for 5 min, followed by 30 cycles of denaturation at 95 ºC for 30 sec, annealing at 58 ºC for 30 sec and extension at 72 ºC for 1 min. The final extension was performed for 5 min at 72 ºC. The PCR products were purified using a FavorPrep Gel/PCR Purification Kit (Favorgen Biotech Pingtung, Taiwan) according to the manufacturer's protocol. The purified PCR products were sequenced by a service provider.

The sequences of the isolates were compared with those in GenBank using the BLAST search. We performed a phylogenetic analysis using Molecular Evolutionary Genetic Analysis (MEGA7) software (Kumar *et al.*, 2016). Datasets with combined ITS regions and β-tubulin sequences were used to generate a maximum likelihood tree, which was constructed using the Kimura 2-parameter with the G+I model (Kimura, 1980). A bootstrap analysis was performed with 1,000 replications to support the classification of each clade. The ITS and β-tubulin sequences of the ex-type specimens of *Penicillium* and *Talaromyces* species available from GenBank were included for comparison.

RESULTS AND DISCUSSION

Based on the microscopic characteristics of the conidiophore branching patterns, conidia shapes and colony appearance, the isolates were tentatively identified as *Penicillium* spp. *Talaromyces* spp. may have been present, as biverticillate conidiophores were also observed. Previously, biverticillate *Penicillium* was classified as the subgenus *Biverticillium* (Visagie *et al.*, 2014). Moreover, both genera have many similar microscopic and macroscopic characteristics (Houbraken and Samson, 2011), which makes morphological identification challenging. Thus, to accurately identify species, identification based on ITS regions and β-tubulin gene sequences was performed, as these two markers are recommended for identifying *Penicillium* species (Visagie *et al*., 2014). The sizes of the ITS region and β-tubulin gene were 600 bp and 500 bp, respectively. The sequence identity percentages determined using the BLAST search are listed in Table 1.

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Table 1: BLAST search results and species identity based on ITS regions and β-tubulin gene sequences of *Penicillium* and *Talaromyces* isolates from beach sand.

Isolates	Species identified	Percentage similarity (%)	
		ITS regions	β-tubulin
Penicillium spp.			
A1S2-D37	*P. citrinum*	*P. citrinum* (100%)	*P. citrinum* (99%)
A1S3-D70	*P. citrinum*	*P. citrinum* (99%)	*P. citrinum* (100%)
A1S4-D52	*P. citrinum*	*P. citrinum* (99%)	*P. citrinum* (100%)
A1S5-D21	*P. citrinum*	*P. citrinum* (99%)	*P. citrinum* (100%)
A3S1-D7	*P. citrinum*	*P. citrinum* (99%)	*P. citrinum* (100%)
A3S6-D1	*P. citrinum*	*P. citrinum* (100%)	*P. citrinum* (99%)
A2S6-6	*P. citrinum*	*P. citrinum* (100%)	*P. citrinum* (100%)
A1S2-D30	*P. oxalicum*	*P. oxalicum* (100%)	*P. oxalicum* (99%)
A1S2-D38	*P. oxalicum*	*P. oxalicum* (100%)	*P. oxalicum* (99%)
A1S1-D35	*P. oxalicum*	*P. oxalicum* (100%)	*P. oxalicum* (99%)
A1S2-D36	*P. oxalicum*	*P. oxalicum* (100%)	*P. oxalicum* (99%)
A1S3-3	*P. oxalicum*	*P. oxalicum* (100%)	*P. oxalicum* (98%)
A2S2-D35	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* (96%)
A3S6-47	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* (96%)
A2S4-D60	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* (96%)
A3S1-14	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* 100%)
A3S1-37	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* (96%)
A3S2-6	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* (96%)
A3S2-20	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* (95%)
A3S3-31	*P. georgiense*	*P. georgiense* (99%)	*P. georgiense* (95%)
A1S4-D37	*P. chermesinum*	*P. chermesinum* (99%)	*P. chermesinum* (100%)
A1S4-D3	*P. chermesinum*	*P. chermesinum* (100%)	*P. chermesinum* (100%)
A1S4-D39	*P. chermesinum*	*P. chermesinum* (99%)	*P. chermesinum* (100%)
A4S2-19	*P. pimiteouiense*	*P. pimiteouiense* (100%)	*P. pimiteouiense* (99%)
A4S2-20	*P. pimiteouiense*	*P. pimiteouiense* (100%)	*P. pimiteouiense* (99%)
A4S2-21	*P. pimiteouiense*	*P. pimiteouiense* (99%)	*P. pimiteouiense* (99%)
A4S2-19	*P. pimiteouiense*	*P. pimiteouiense* (100%)	*P. pimiteouiense* (99%)
A1S4-D12	*P. daleae*	*P. daleae* (99%)	*P. daleae* (98%)
A1S4-D42	*P. daleae*	*P. daleae* (99%)	*P. daleae* (98%)
A4S6-1	*P. flaviroseum*	*P. flaviroseum* (99%)	*P. flaviroseum* (99%)
A1S3-D87	*P. rolfsii*	*P. rolfsii* (99%)	*P. rolfsii* (98%)
A1S5-D11	*Penicillium* sp.	*P. cluniae* (84%)	*P. cluniae* (85%)
A1S5-D20	*Penicillium* sp	*P. cluniae* (84%)	*P. cluniae* (85%)
Talaromyces spp.			
A1S5-D31	*T. siamense*	*T. siamense* (99%)	*T. siamensis* (98%)
A2S1-D75	*T. siamense*	*T. siamense* (99%)	*T. siamensis* (100%)
A3S2-39	*T. siamense*	*T. siamense* (98%)	*T. siamensis* (98%)
A3S2-40	*T. siamense*	*T. siamense* (98%)	*T. siamensis* (98%)
A2S3-4	*T. atroroseus (*99%)	*T. atroroseus (*99%)	*T. atroroseus (*99%)
A3S2-11	*T. atroroseus (*99%)	*T. atroroseus* (99%)	*T. atroroseus (*99%)
A3S3-9	*T. atroroseus (*99%)	*T. atroroseus* (99%)	*T. atroroseus (*99%)
A3S3-40	*T. atroroseus (*99%)	*T. atroroseus* (99%)	*T. atroroseus (*99%)
A4S1-66	*T. atroroseus (*99%)	*T. atroroseus* (99%)	*T. atroroseus (*99%)
A4S1-88	*T. atroroseus (*99%)	*T. atroroseus* (99%)	*T. atroroseus (*99%)
A2S6-3	*T. fusiformis* (100%)	*T. fusiformis* (100%)	*T. fusiformis* (100%)

The maximum likelihood phylogenetic tree of the combined ITS region and β-tubulin gene sequences of *Penicillium* and *Talaromyces* isolates from the beach sand formed two main clades (I and II) and 13 sub-clades (1-13). The isolates of the same species were clustered together with the type strains and formed separate clades (Figure 1). Based on the closest match of the BLAST search and phylogenetic analysis, nine *Penicillium* species (*P. pimiteouiense*, *P. chermesinum*, *P. citrinum*, *P. oxalicum*, *P. daleae*, *P. flaviroseum*, *P. rolfsii*, *P. georgiense* and *Penicillium* sp.) and four *Talaromyces* species (*T. siamense*, *T. minioluteus*, *T. fusiformis* and *T. astroroseus*) were identified. There were *Penicillium* isolates that could not be identified to the species level but were related to *P. simplicissimum*, based on the phylogenetic analysis. The microscopic characteristics of

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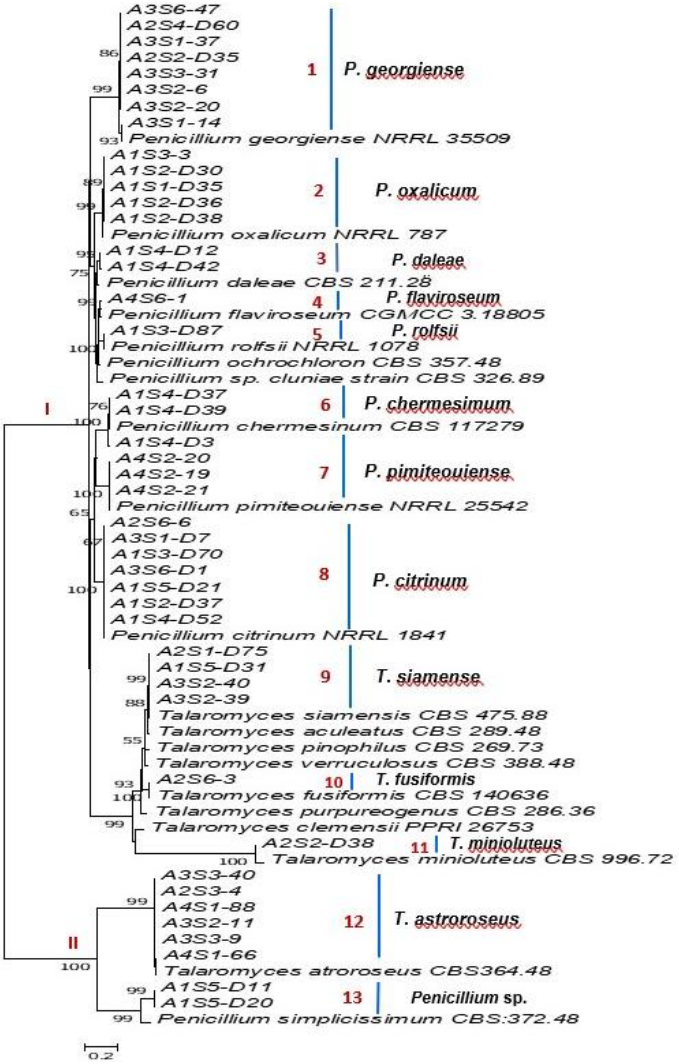

Figure 1: Maximum likelihood tree of *Penicillium* and *Talaromyces* isolates from beach sand generated based on combined sequences of ITS regions and β-tubulin gene.

Penicillium and *Talaromyces* isolates are presented in Table 2. Figures 2 and 3 show the conidiophore and conidia of the *Penicillium* and *Talaromyces* species identified in the present study. Table 3 shows the colony characteristics of the *Penicillium* and *Talaromyces* isolates identified and the colony appearance on several differential media are shown in Figures 4, 5 and 6.

There are several reports on the occurrence of *Penicillium* on various tropical beaches around the world. de Moura Sarquis and de Oliveira (1996) reported that the genus *Penicillium* from the sandy soil of Ipanema Beach, Rio de Janeiro, Brazil had more species (11 species) compared with other microfungal genera. Pinto *et al*. (1992) and Migahed (2003) also reported *Penicillium* species was among the most common genera isolated from Boa Viagem Beach, Brazil and several beaches in Egypt, respectively. *Penicillium* was one of the most frequently isolated genera from sand in the Casa Caiada and Bairro Novo beaches, Brazil (Gomes *et al.*, 2008) and four sandy beaches along the Algerian western coastal area (Matallah-Boutiba *et al.*, 2011).

In the present study, *P. georgiense* and *P. citrinum* were the most common species isolated from the beach sand. *Penicillium georgiense* was first isolated from peanut field soils in Georgia, USA, where the fungus grew and developed on the conidial heads of *Aspergillus* section Nigri (Peterson and Horn, 2009). The prevalence of *P. georgiense* in the beach sand may be associated with the abundance of black Aspergilli in the beach soil environment, as reported by Teh and Latiffah (2015). *Penicillium citrinum* was among the species of *Penicillium* isolated from beach sand in Boa Viagem Beach, Brazil (Pinto *et al.*, 1992), Ipanema Beach, Rio de Janeiro (de Moura Sarquis and de Oliveira, 1996) and from several Egyptian beaches (Migahed, 2003). *Penicillium citrinum* frequently occurs in tropical and subtropical soils and

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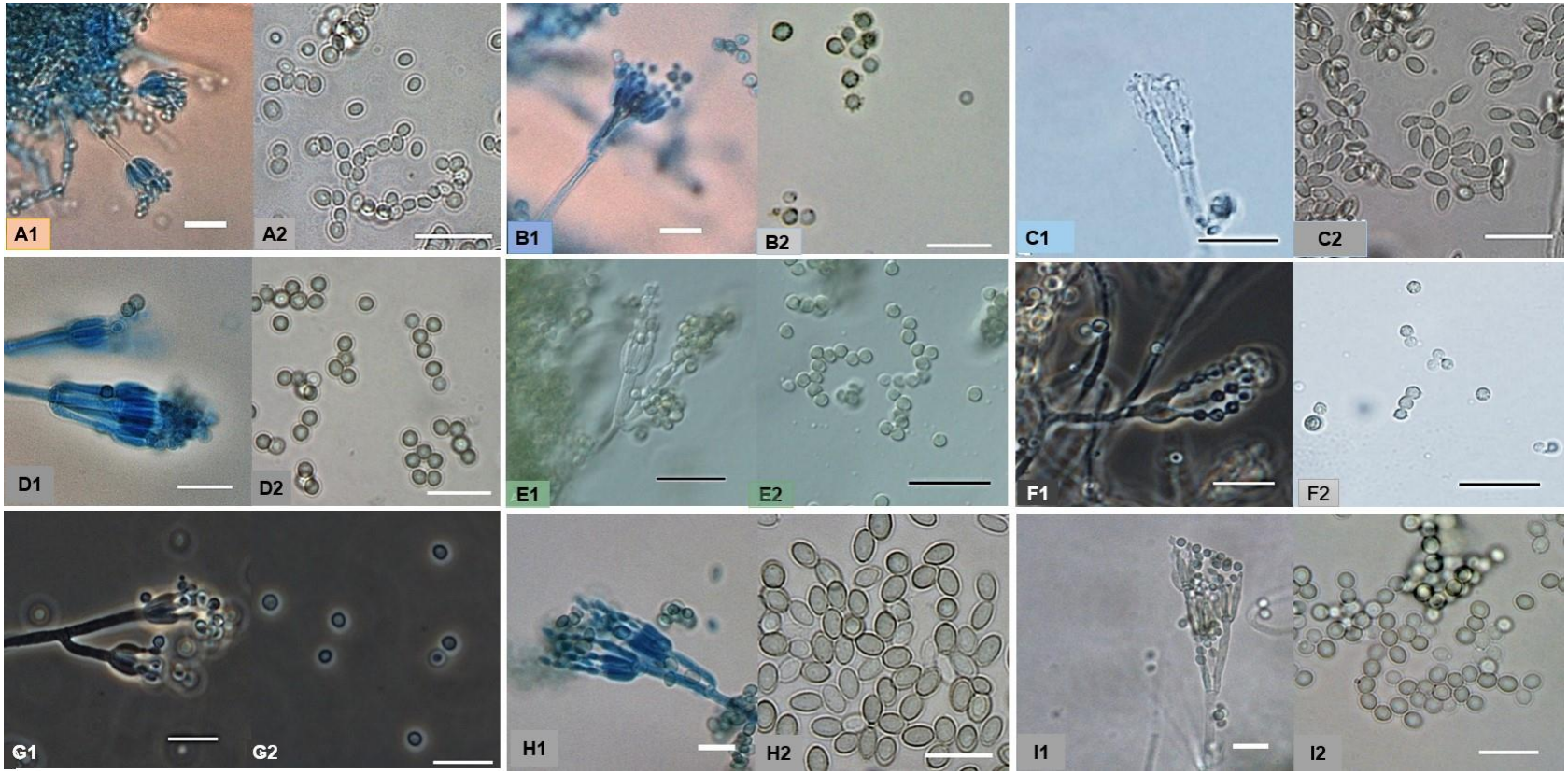

Figure 2: Conidiophore and conidia of *Penicillium* species isolated from beach sand. A1, A2: *P. chemesimum*; B1, B2: *P. flaviroseum*; C1, C2: *P. roflsii*; D1, D2: *P. citrinum*; E1, E2: *P. georgiense*; F1, F2: *P. pimitouense*; G1, G2: *P. daleae*; H1, H2: *P. oxalicum;* I1, I2: *Penicillium* sp.

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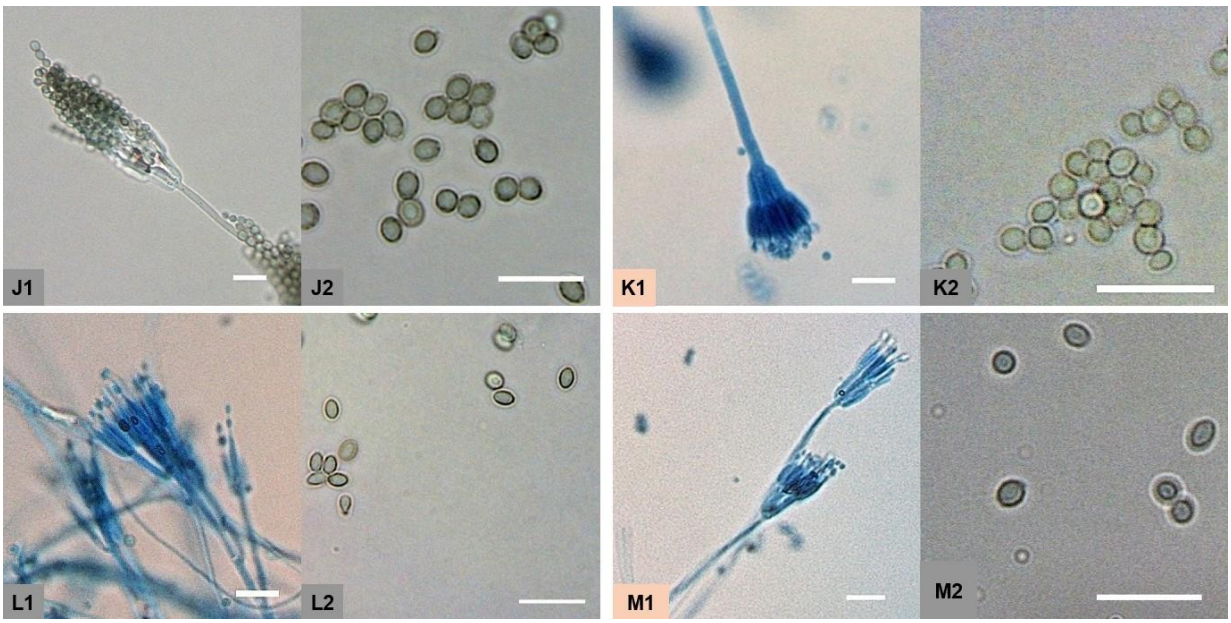

Figure 3: Conidiophore and conidia of *Talaromyces* species isolated from beach sand. J1, J2: *T. astroroseus*; K1, K2: *T. minioluteus*; L1, L2: *T. fusiformis*; M1, M2: *T. siamense*.

Table 2: Microscopic characteristics of *Penicillium* and *Talaromyces* isolates from beach sands.

Species	Conidia			Conidiophore	
	Diameter (µm)	Ornamentation	Shape	Branching pattern	Stipe ornamentation
Penicillium					
P. citrinum	1.9-3.0	Smooth	Globose	Biverticillate	Smooth
P. flaviroseum	2.2-3.6	Spinose	Globose	Biverticillate	Smooth
P. georgiense	2.0-3.3	Smooth	Globose	Monoverticillate	Smooth
P. daleae	2.1-3.1	Finely roughened	Globose	Monoverticillate	Smooth
P. pimiteouiense	2.0-3.2	Spinose	Globose	Monoverticillate	Smooth
Penicillium sp.	2.6-4.2	Smooth	Ellipsoidal	Monoverticillate	Smooth
P. oxalicum	2.6-5.8	Smooth	Ellipsoidal	Monoverticillate	Smooth
P. chermesinum	1.8-2.7	Smooth	Subglobose to ellipsoidal	Monoverticillate	Smooth
P. purpurogenum	2.6-3.8	Smooth	Subglobose to ellipsoidal	Biverticillate	Smooth
Talaromyces					
T. siamense	1.7-3.2	Smooth	Subglobose to ellipsoidal	Biverticillate	Smooth
T. fusiformis	2.1-3.8	Smooth	Subglobose to ellipsoidal	Biverticillate	Smooth
T. minioluteus	1.7-2.8	Smooth	Subglobose	Biverticillate	Smooth
T. astroroseus	2.8-3.7	Spinose	Globose	Biverticillate	Smooth

grows at 37 °C (Houbraken and Samson, 2011), indicating that beach sand is among the substrates occupied by this fungus.

The other *Penicillium* species have also been isolated from various soils worldwide in different studies, indicating their occurrence as soil fungi: *P. flaviroseum* (Diao *et al.*, 2019), *P. rolfsii* (Lee *et al.*, 2016), *P. daleae* (Cho *et al.*, 2005; Szewczyk, 2007), *P. oxalicum* (Sabuquillo *et al.*, 2006) and *P. chemisimum* (Singh *et al.*, 1985).

Although four *Talaromyces* species were identified in the present study and many *Talaromyces* species are common soil inhabitants, there is only one report on the occurrence of *Talaromyces* in beach sand, which was from Candeias Beach, Pernambuco, Brazil (de Oliveira *et al.*, 2011). This may be due to the genus being recognised as a teleomorph of *Penicillium* before it was redefined by Samson *et al.* (2011). Another possible reason is many *Talaromyces* species are not easily

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Table 3: Colony characteristics of *Penicillium* and *Talaromyces* species on differential media.

Species	Colony on MEA			Colony on CYA			Colony on YES			Colony on CREA		Ehrlich
	Diameter (mm)	Upper colour	Lower colour	Diameter (mm)	Upper colour	Lower colour	Diameter (mm)	Upper colour	Lower colour	Growth	Acid	
P. citrinum	23-30	Dark green	Cream	27-33	Dark green	Orange	27-47	White to dark green	Orange	Poor	+	No reaction
P. oxalicum	28-44	Dark green	Yellow	21-32	Dull green	Cream	52-60	Greenish-white	Yellow	Poor	+	Violet
P. georgiense	18-25	Dark green	Olive green	13-19	White	Cream	13-17	Green	Brown	Poor	-	No reaction
P. chermesinum	26-32	Grey	Yellow	28-32	Grey	Yellow	30-38	White	Yellow	Poor	++	No reaction
T. siamense	27-40	Greenish-white	Orange	27-31	Greyish-green	Yellow	28-34	Red	Red	Poor	-	No reaction
T. astroroseus	22-38	Blue-green	Red	32-37	Blue-green	Red	38-44	Blue-green	Red	Poor	+	No reaction
P. pimiteouiense	21-30	White	Orange	20-24	White	Orange	25-31	White	Orange	Poor	-	No reaction
P. daleae	38-47	Greyish-white	Yellow	40-48	White	Yellow	39-50	White	Yellow	Poor	-	No reaction
P. rolfsii	55-57	Greenish-grey	Yellow	57-59	Grey	Yellow	68-70	White	Yellow	Poor	+	No reaction
P. verruculosum	30-31	Greenish-white	Cream	34	Greenish-white	Cream	30-32	Brownish-white	Cream	Poor	+	No reaction
T. fusiformis	25-32	Yellowish-green	Orange	29-30	Yellow	Orange	28-29	White	Cream	Poor	+	No reaction
T. minioluteus	17-20	Yellowish-green	Orange	17-19	Yellow green	Dark Orange	16-20	Greenish	Orangy	No growth	-	No reaction
P. flaviroseum	18-38	Greyish-green	Cream	39-41	Greenish-white	Pink	56-57	White with pink ring	Yellow	Poor	++	No reaction

Acid production: - no acid production, + weak acid production, ++ good acid production.

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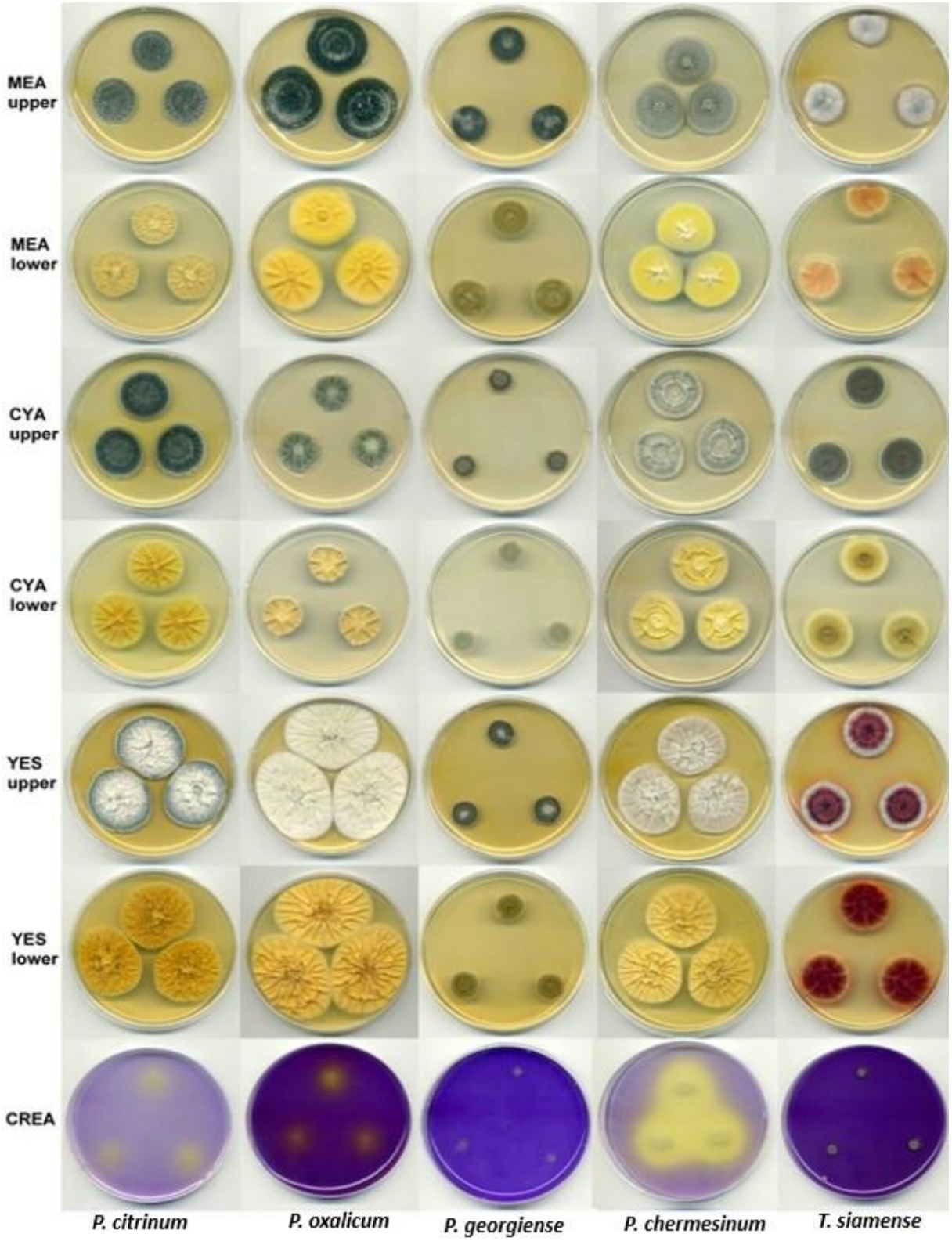

Figure 4: Colony morphology of *P. citrinum*, *P. oxalicum*, *P. georgiense*, *P. chermesinum* and *T. siamense* on MEA, CYA, YES and CREA.

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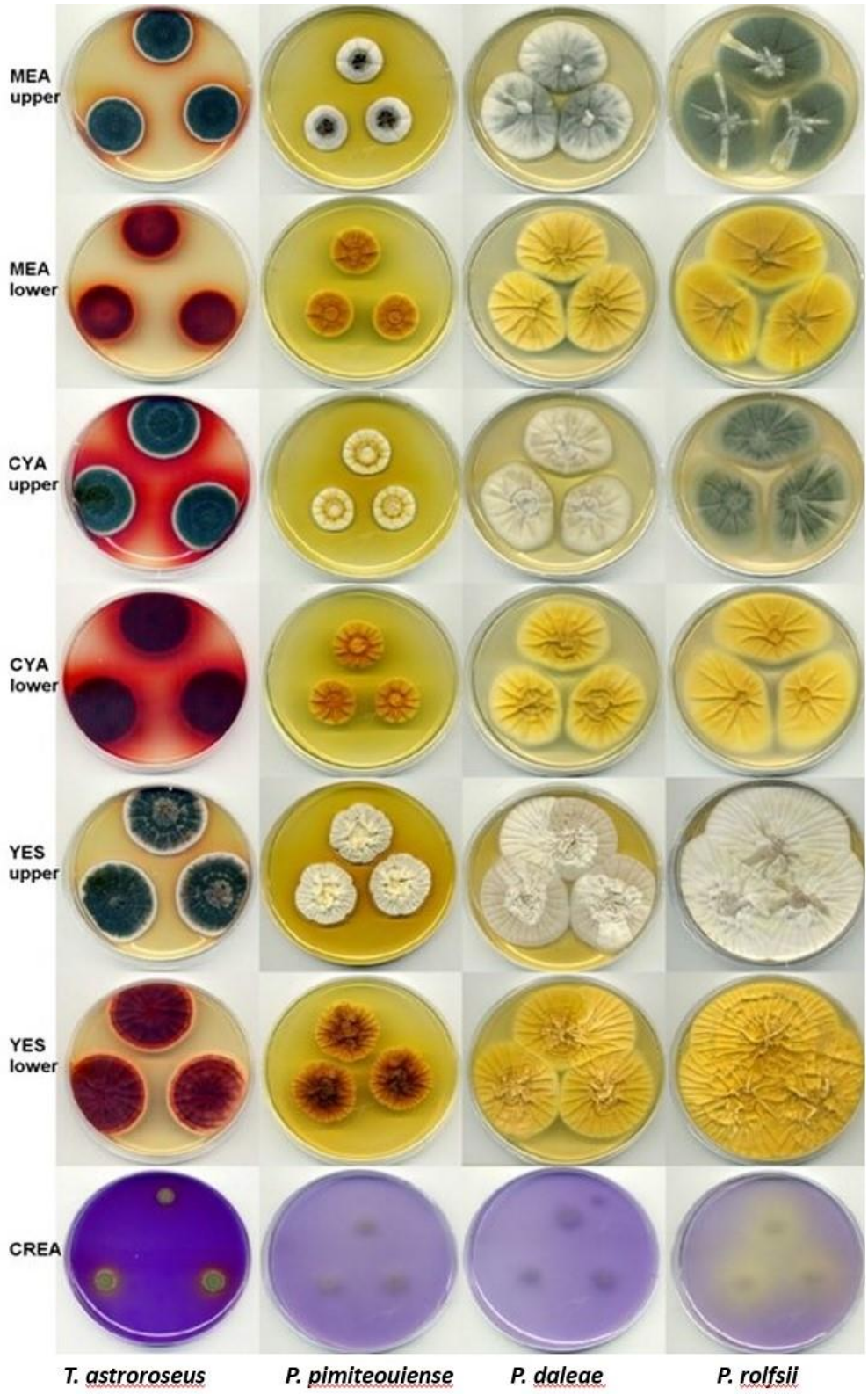

Figure 5: Colony morphology of *T. astroroseus*, *P. pimiteouiense*, *P. daleae* and *P. rolfsii* on MEA, CYA, YES and CREA.

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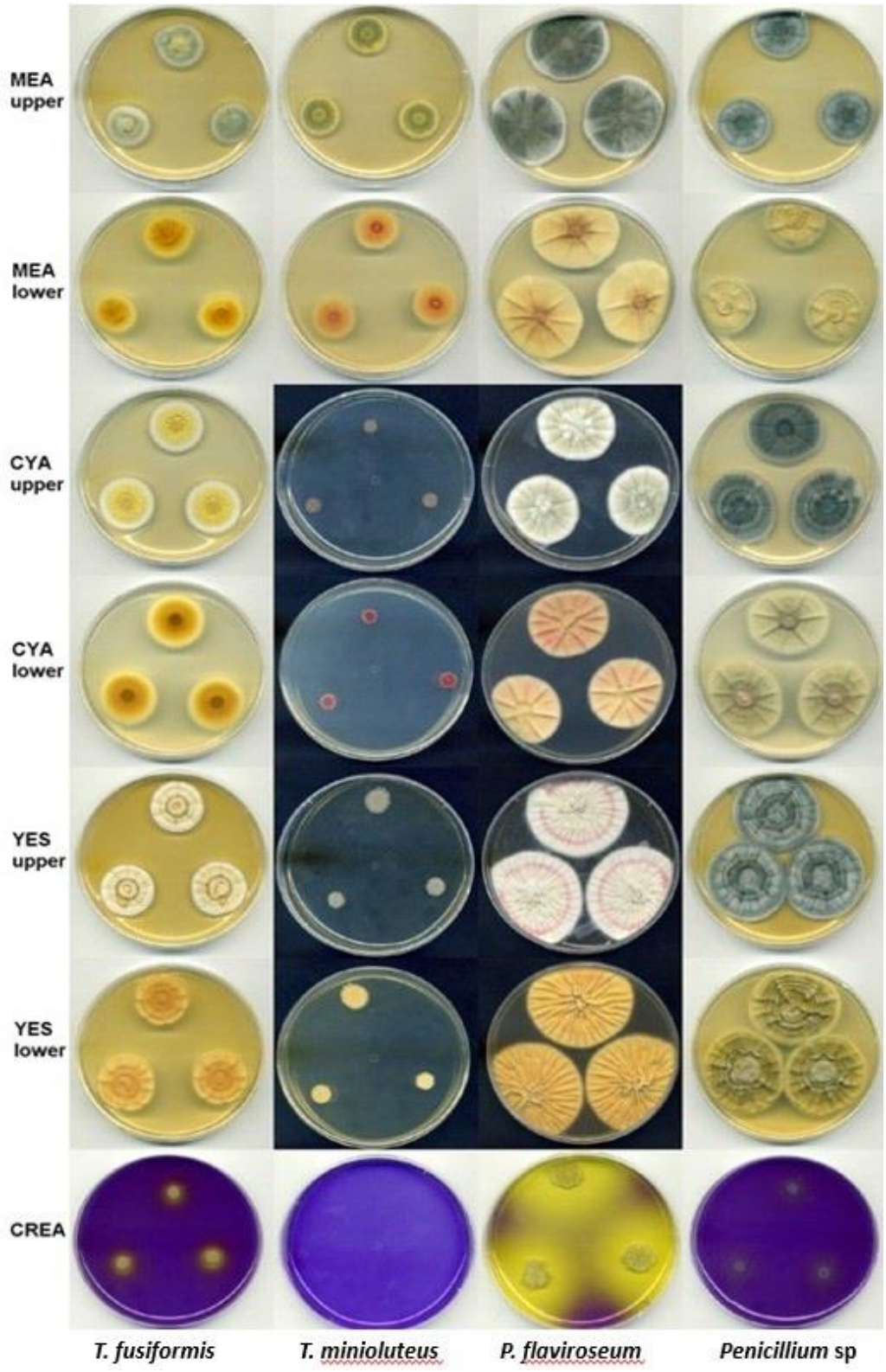

Figure 6: Colony morphology of *T. fusiformis*, *T. minioluteus*, *T. flaviroseum* and *Penicillium* spp. on MEA, CYA, YES and CREA.

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isolated, as some species cannot grow well on artificial media or grow very slowly and are easily overgrown by faster-growing fungi (Pitt, 1979; Wei *et al.*, 2021).

Among the four *Talaromyces* species recovered from beach sand, *T. astroroseus*, *T. minioluteus* and *T. siamense* have been isolated from soils (Manoch and Ramirez, 2004; Frisvad *et al.*, 2013; Lima *et al.*, 2018). Only *T. fusiformis* was not isolated from the soil but was found in an indoor environment (Chen *et al.*, 2016).

Although *Penicillium* and *Talaromyces* are often found as saprophytes, many species are opportunistic human pathogens, mainly infecting immunocompromised individuals. In clinical settings, *Penicillium* spp. are associated with allergies as well as superficial and invasive infections (Lyratzopoulos *et al.*, 2002). Among the *Penicillium* species identified in the present study, *P. citrinum* and *P. oxalicum* have been reported as opportunistic human pathogens (Chowdhary *et al.*, 2014; Hesse *et al.*, 2017). Another species, *P. pimiteouiense* was isolated from epithelial cells in polycystic kidney disease, but the role of the fungus was not known (Peterson *et al.*, 1999). Regarding *Talaromyces, T. atroroseus* was isolated from lung samples (Guevara-Suarez *et al*., 2016). So far, the other three *Talaromyces* spp. have not been associated with human infections. The most notable human pathogenic species of *Talaromyces* is *T. marneffie*, which causes systemic mycosis in HIV-infected individuals (Chitasombat and Supparatpinyo, 2013). Opportunistic species of *Penicillium* and *Talaromyces* infect immunocompromised individuals through the inhalation of conidia or spores or direct contact with sands that contain them.

The dispersal of *Penicillium* and *Talaromyces* occurs mainly through spores or conidia, and viable fungal conidia transmit the fungal infection to humans (Mancini *et al.*, 2005). Fungal spores or conidia survive between 25 and 360 days in a beach environment and environmental factors such as the physical characteristics of the beach, tides, sewage outlets and human activities can promote the survival and dispersal of the conidia or spores on beach sand (Mendes *et al.*, 1998; World Health Organization, 2003). Other environmental factors that may affect the presence of viable conidia are rain, relative humidity, turbulence and gravity forces (Larrondo and Calvo, 1989).

CONCLUSION

Based on ITS and β-tubulin sequences, nine *Penicillium* spp. and four *Talaromyces* spp. were identified. The *Penicillium* spp. were identified as *P. georgiense*, *P. chermesinum*, *P. pimiteouiense*, *P. citrinum*, *P. oxalicum*, *P. daleae*, *P. rolfsii* and *Penicillium* sp., and the four *Talaromyces* spp. were *T. siamense*, *T. atroroseus*, *T. minioluteus* and *T. fusiformis*. The occurrence of *Penicillium* and *Talaromyces* in beach sand is likely due to the accumulation of organic matter generated by human activities and the presence of suitable environmental conditions for the viability and survival of both microfungi. The present study contributes to our knowledge of the biodiversity and systematics of *Penicillium* and *Talaromyces* species in the beach sand of Malaysia.

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