

Filamentous ascomycete and basidiomycete fungi from beach sand

Teh Li Yee¹ · Rosnida Tajuddin¹ · Nik Mohd Izham Mohamed Nor¹ ·
Masratul Hawa Mohd¹ · Latiffah Zakaria¹

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Abstract The beach environment harbours filamentous fungi from several genera that played an important role as primary degraders of organic substrates in the beach ecosystem. In this study, filamentous fungi were isolated from beach sand collected along Batu Ferringhi and Teluk Bahang beach areas, Penang Island, Peninsular Malaysia. A total of 260 isolates of filamentous fungi were isolated and identified based on ITS sequences. Based on the closest match of BLAST search, 90 sequences were identified to species level. Ascomycetes showed higher occurrences in the beach sand samples compared to basidiomycetes. Only seven species of basidiomycetes were recovered from the beach sand samples. The total number of filamentous fungal genera identified was 50 with highest occurrences of *Aspergillus* (39.6 %) and *Penicillium* (17.6 %). Filamentous fungi from other genera were detected at a relatively low occurrences ranging from 0.3 to 3.4 %. The present study showed that beach sand harbours a variety of filamentous fungi which can be saprotrophic and potentially pathogenic. This study contributes to the knowledge on the occurrence and diversity of filamentous fungi in beach sand in Malaysia providing information for future researches that involve human, marine, and plant pathology.

Keywords Filamentous fungi · Occurrence · Diversity · Beach sand · ITS region

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✉ Latiffah Zakaria
Lfah@usm.my; latiffahz@yahoo.com

¹ School of Biological Sciences, Universiti Sains Malaysia (USM), 11800 Penang, Malaysia

1 Introduction

Beaches are being supplied with large amounts of organic matter by the sea water, plants on the seashore as well as frequent human and animal activities. The accumulation of these organic matters provided the suitable conditions for the growth of heterotrophic microorganisms including filamentous fungi, which play an important role in the degradation processes of organic matter accumulated in the sandy beach ecosystems (Mudryk and Podgorska 2007).

In general, microbial communities in beach sand have received relatively little attention compared to those in water and submerged sediment. Most of the publications related to filamentous fungi in marine and coastal ecosystems are referred to Europe, North America and South America regions (Dunn and Baker 1984; Gomes et al. 2008; Sabino et al. 2011). Moreover, the information of the mycota in beach sand in Malaysia is lacking especially fungi potentially pathogenic to human and animals.

Fungi exist abundantly in the soil environment and play an important role as primary decomposers of organic materials. Many soil fungi are also potential pathogens to plants, animals and human. The majority of fungi in beach sand might be in resting stages existing as dormant spores and mycelia fragments in the sand. The diversity of fungi in beach sand can be affected by contamination and erosion due to human activities and thus this study is important to gain knowledge on the diversity of fungi in the beach frequently visited by beachgoers.

With the development of sequence analysis, ITS region is widely applied in molecular ecology of fungi (Peay et al. 2008) and has been recommended as the universal fungal barcode for rapid identification of fungi (Schoch et al. 2012). The region is also useful for molecular systematics at the interspecific level, as well as intraspecific level.

James et al. (1996) demonstrated that ITS region is useful in determining sequence variation between closely related species as the region has higher rates of divergence than 18S or 26S rRNA genes.

Although the tropical beaches in Malaysia provide an ideal habitat for a wide diversity of filamentous fungi, no studies have been published using molecular approaches for the identification of filamentous fungi isolated from beach sand. Moreover, the information of potential humans and animals fungal pathogens in beach sand is lacking. Therefore, the objective of this study was to determine the occurrence and identify filamentous fungi from beach sand in Malaysia using ITS sequences.

2 Materials and methods

Twenty-four soil samples were collected along the beaches of Batu Ferringhi and Teluk Bahang, Penang Island, Peninsula Malaysia (Online Resource T1) of which 12 soil samples were collected from each beach. The soils were taken by scraping off the surface and subsurface to a depth of 10 cm. All the soil samples were air-dried at 27 ± 1 °C for 48–72 h. The dried soil samples were sieved through a 2.0-mm sieve to remove stones and plant residues or plant debris.

Isolation of filamentous fungi was done using direct, dilution and debris isolation techniques. Malt Extract Agar (MEA) incorporated with streptomycin and neomycin was used as isolation medium. For direct isolation, 0.3 g of soil sample was distributed evenly on MEA. In soil dilution technique, 1 g of air-dried soil was put into a Universal bottle containing 10 ml sterile water and mixed well for preparation of soil suspension with 10^{-1} dilution. The steps were repeated until soil suspension with 10^{-4} dilution. One millilitre of the soil suspension from each dilution was transferred and evenly distributed on MEA with a spreader. Debris collected during sampling and debris remaining on the sieves were washed under running tap water for 24 h. After washing, the debris were dried over a filter paper and cut into small pieces (10–15 mm) and transferred onto MEA. For the three isolation techniques, the MEA plates were incubated for 5–7 days at 27 ± 1 °C until the growth of fungal colonies was observed.

Mycelia for DNA extraction were grown in Universal bottles with Potato Dextrose Broth (PDB) at 25 °C. Mycelia were harvested by filtration when mycelium was visible with no sporulation, generally after 16–48 h. Mycelia were frozen and lyophilized, and then crushed using liquid nitrogen. Genomic DNA was extracted using Invisorb® Spin Plant Mini Kit (Strattec Molecular GmbH, Berlin) according to the manufacturer's protocol. The ITS

regions were amplified using ITS 1 and ITS 4 primers (White et al. 1990).

The PCRs for ITS regions were performed in TM Peltier Thermal Cycler Model PTC-100 (MJ Research Watertown, MA, USA). DNA amplifications were performed in a total volume of 25 µl containing 0.5 µl of genomic DNA, 4.0 mM MgCl₂, 0.8 mM dNTPs (Promega Madison, WI, USA) and 0.625 U of Taq polymerase (Promega Madison, WI, USA) with 0.5 µM primer concentration. PCR cycles started with an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 30 s denaturation at 95 °C, 30 s annealing at 58 °C and 1 min extension at 72 °C. Final extension for 5 min at 72 °C was performed after the cycles ended. PCR products were separated by electrophoresis on 1 % agarose gels and checked to ensure that a single DNA band of desired size was produced. PCR products were purified using FavorPrep™ Gel/PCR Purification Kit (Favorgen® Biotech Corp., Pingtung, Taiwan) according to the manufacturer's protocol. The purified products were sent to a service provider for DNA sequencing.

The sequences of filamentous fungal isolates were compared with sequences in the GenBank using BLAST. All the ITS regions sequences of filamentous fungi isolates were deposited in GenBank.

3 Results

Two hundred sixty isolates were isolated and identified based on the closest match of sequences of ITS region in GenBank. The size of the PCR products was approximately 600 bp. The percentage of similarity from BLAST search and accession numbers of the sequences are listed in Online Resource T2. Based on the closest match, all the isolates showed the percentage of similarity ranging from 95 to 100 %.

The BLAST search results showed a high number of ascomycetes isolated from the beach sand samples compared to basidiomycetes. Seven species of basidiomycetes were recovered: *Perenniporia tephropora*, *Ceriporia lac-erata*, *Phanerochaete chrysosporium*, *Coprinellus radians*, *Coprinellus disseminatus*, *Coprinopsis cinerea* and *Cori-olopsis polyzona*.

Ascomycetes from the genus *Aspergillus* (103 isolates) were the most commonly isolated filamentous fungi from the beach sand followed by *Penicillium* (46 isolates), *Trichoderma* (9 isolates), *Fusarium* (8 isolates), *Hypoxyton* (8 isolates), *Xylaria* (8 isolates), *Phoma* (6 isolates), *Chaetomium* (5 isolates), *Paraconiothyrium* (5 isolates), *Robil-larda* (4 isolates), *Curvularia* (4 isolates), *Engyodontium* (4 isolates), *Pestalotiopsis* (4 isolates), *Humicola* species (2

isolates), *Nigrospora* (2 isolates) and other filamentous fungi as shown in Online Resource T2.

Five species of Hyphomycetes were identified, namely *Microdochium nivale*, *Graphium penicillioides*, *Hansfordia sinuosae*, *Arthrimum phaeospermum* and *Periconia macrospinoso*. Six species from the class Sordariomycetes were also recovered and identified as *Bartalinia pondoensis*, *Daldinia eschscholtzii*, *Neurospora calospora*, *Valsa brevispora*, *Bionectria epichloe* and *Myrothecium inundatum*. Majority of the ascomycetes (18 isolates) were from the Class Dothideomycetes.

4 Discussion

In Malaysia, sandy beaches are often sought after by locals and tourists for recreational purposes and the present study showed there is significant presence of saprotrophic and potentially pathogenic filamentous fungi. Recreational activities at the beach which involve sitting, lying, strolling and building sand castles may pose health risks for some beachgoers especially infection from opportunistic pathogens (Whitman et al. 2014). It was suggested that the higher frequency of human and animal activities could contribute to the occurrence of filamentous fungi in beach sand (World Health Organization 2003; Mancini et al. 2005).

Filamentous fungi have been isolated from several beaches worldwide (Larrondo and Calvo 1989; Oliveira et al. 2011). Factors such as the nature of the beach, tidal phenomena, sewage outlets, seasons, the presence of animals and the number of bathers, can encourage the survival and dispersion of pathogens on beach sand (World Health Organization 2003).

Some filamentous fungi are common saprophytes in the beach environment; however, they may act as opportunistic pathogens, especially on immunocompromised patients (de Hoog et al. 2000). Moreover, the viable fungal conidia can act as an agent in the transmission of fungal infection in humans (Larrondo and Calvo 1989). Several species of *Aspergillus* have been reported to be potentially pathogenic to humans and animals. *Aspergillus fumigatus* and non-fumigatus species have been associated with aspergillosis which is a common fungal infection of immunocompromised individuals (Pitt 1994). Members of the genus *Trichoderma* such as *Trichoderma longibrachiatum* have been known to cause invasive infections on immunocompromised patients which could result in fatality if severely infected on a bone marrow (Richter et al. 1999). According to Cortez et al. (2008), *Scedosporium* species have become increasingly recognized as an emerging pathogen amongst immunocompromised individuals. *Lasiodiplodia theobromae* (= *Botryodiplodia theobromae*) has been reported to

cause subcutaneous phaeohyphomycosis and keratitis in humans (Sutton 1999). The first case of mycosis in a healthy man caused by *Arthrimum phaeospermum* var. *indicum* has been reported by Rai (1989). *Fusarium oxysporum* has been isolated from sea turtle nests in eastern Australia as well as the exterior of failed eggs of sea turtles (Phillott et al. 2004). The results of the present study suggest that the beaches' sands harbour a variety of filamentous fungi with opportunistic pathogenic activity on animal and humans.

Amongst the filamentous fungi, species from the genera *Aspergillus* and *Penicillium* were more frequently isolated compared to the other genera. The predominance of these fungal genera have also been observed in sandy soils of Ipanema Beach, Rio de Janeiro (Moura Sarquis and Oliveira 1996); the beach sand from coastal beaches located on the coasts of the Caribbean Sea, Gulf of Mexico, the Pacific Ocean, Bairro Novo, Casa Caiad and Candeias, Pernambuco (González et al. 1998; Gomes et al. 2008; Oliveira et al. 2011). The data obtained show a much higher species diversity of *Aspergillus* and *Penicillium* than reported by González et al. (1998) and Oliveira et al. (2011).

Many of the filamentous fungal species isolated in the present study have also been reported from various beaches in Brazil. Eight species isolated in the present study, namely *Fusarium oxysporum*, *Fusarium proliferatum*, *Trichoderma harzianum*, *Trichoderma longibrachiatum*, *Paecilomyces variotii*, *Cladosporium cladosporioides*, *Pestalotiopsis disseminata* and *Humicola fuscoatra* have also been isolated from Ipanema Beach in Rio de Janeiro (Moura Sarquis and Oliveira 1996). Six species, *Fusarium oxysporum*, *Cladosporium cladosporioides*, *Paecilomyces variotii*, *Trichoderma harzianum*, *Chaetomium globosum* and *Lasiodiplodia theobromae* have also been isolated and identified from sand samples collected from Casa Caiada beach and Bairro Novo beach in Olinda (Gomes et al. 2008). *Phoma sorghina*, *Trichoderma harzianum*, *Microsphaeropsis arundinis* and *Cladosporium cladosporioides* have been reported from Candeias beach, Pernambuco (Oliveira et al. 2011).

In Catalonia, Spain, three fungal species, *Phoma herbarum*, *Fusarium oxysporum* and *Cladosporium cladosporioides*, have also been recovered from sandy beaches of the Mediterranean coast of Catalonia (Larrondo and Calvo 1989). Six fungal species from the present study, *Cladosporium cladosporioides*, *Nigrospora sphaerica*, *Curvularia tuberculata*, *Curvularia lunata*, *Graphium penicillioides*, *Chaetomium globosum* and *Lasiodiplodia theobromae*, have also been reported in sandy soil from three beaches in Mexico (González et al. 1998).

The basidiomycetes could exist in the beach sands in the form of basidiospores or detached mycelia fragments. If

suitable substrate is present, the basidiomycetes will form mycelial cords that transport nutrient and water as well as acquire inorganic nutrients from soil, hence allowing basidiomycetes colonies to grow in nutrient-depleted soil (Wells et al. 1997). Amongst the basidiomycetes, *Ceriporia lacerata*, a white rot fungus has been implicated with respiratory diseases (Chowdhary et al. 2013).

Mycoflora communities in beach environment received relatively little attention although World Health Organization (2003) has highlighted that the quality of beach sediments is important as the sediment may contain potential microbiological contamination. Studies on the quality of beach sand in Italy and Portugal have also highlighted the potential of sanitary hazards to humans from fungal and other microbial contamination (Mancini et al. 2005; Soussi et al. 2007; Pereira et al. 2013; Sabino et al. 2011). From this study, fungal communities at beaches are diverse and consist of potential health hazardous fungal groups.

Although there is no correlation reported between the occurrence of pathogenic fungi in beach sands and health risk to beachgoers, the exposure to sand containing fungal pathogens increased risk of direct contact with skin and mucous membranes or by inhaling spores as many filamentous fungi contain substances in their spores that can cause symptoms of irritation and allergic reaction (Matavulj et al. 2005; Sabino et al. 2011). Due to potential health risk from potentially harmful and pathogenic fungi in beach environment, Sabino et al. (2011) have conducted a study on microbiological quality of sand on selected beaches in Portugal and proposed a threshold value of 17 cfu/g for potential pathogenic fungi. The threshold value is useful baseline for assessment of sand beach microbiological flora and as a warning to the occurrence of potentially harmful fungi (Sabino et al. 2011). Recently, Solo-Gabriele et al. (2016) suggested that microbes in beach sand including fungi have the potential to be infectious in disease transmission and recommended that microbial measurements in sand to be included in beach monitoring programmes.

5 Conclusion

The present study indicates that beach sands represent a large reservoir of potential pathogenic and allergenic fungi which might be important for plants and animals in the coastal ecosystem. The presence of various filamentous fungi in the beach sand warranted further studies for its potential used as bioindicators to beach contamination, and the potential risks to the beach users' health. Thus, this study extends our current knowledge on the occurrence and diversity of filamentous fungi in the beach soil in Malaysia

which would inform us of the possible pathogenic fungi that may affect human and plants.

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Corresponding author:

*Latiffah Zakaria, School of Biological Sciences, Universiti Sains Malaysia (USM)
11800 Penang, Malaysia

E-mail: Lfah@usm.my / latiffahz@yahoo.com

Online Resource T1 Map of Teluk Bahang and Batu Ferringhi beaches, Penang Island showing the location of the sampling sites (Area 1 to Area 4).



Online Resource T2 Identity of filamentous fungal isolates from beach sand based on percentage of sequence similarity of ITS region

No.	Code	Closest match / Similarity (%)	Accession number
1	A1S3-D97	<i>Aspergillus niger</i> (99%)	JX291201
2	A1S5-D33	<i>Aspergillus niger</i> (100%)	JX291199
3	A1S6-13	<i>Aspergillus niger</i> (99%)	JX501365
4	A1S6-D20	<i>Aspergillus niger</i> (100%)	JX291200
5	A2S1-D105	<i>Aspergillus niger</i> (99%)	JX501371
6	A2S2-2	<i>Aspergillus niger</i> (100%)	JX501376
7	A3S4-D6	<i>Aspergillus niger</i> (100%)	JX291197
8	A4S2-1	<i>Aspergillus niger</i> (100%)	JX291198
9	A1S1-D72	<i>Aspergillus tubingensis</i> (100%)	JX291183
10	A1S4-D31	<i>Aspergillus tubingensis</i> (100%)	JX291184
11	A1S5-15	<i>Aspergillus tubingensis</i> (100%)	JX291185
12	A2S1-7	<i>Aspergillus tubingensis</i> (99%)	JX501370
13	A2S1-D59	<i>Aspergillus tubingensis</i> (100%)	JX291187
14	A2S1-D60	<i>Aspergillus tubingensis</i> (99%)	JX291169
15	A2S2-D32	<i>Aspergillus tubingensis</i> (100%)	JX291194
16	A2S3-2	<i>Aspergillus tubingensis</i> (100%)	JX291192
17	A2S3-D5	<i>Aspergillus tubingensis</i> (100%)	JX291188
18	A2S4-1	<i>Aspergillus tubingensis</i> (100%)	JX291186
19	A2S5-3	<i>Aspergillus tubingensis</i> (100%)	JX501390
20	A2S5-D40	<i>Aspergillus tubingensis</i> (99%)	JX291189
21	A2S5-D88	<i>Aspergillus tubingensis</i> (100%)	JX291190
22	A2S6-7	<i>Aspergillus tubingensis</i> (99%)	JX291193
23	A3S1-2	<i>Aspergillus tubingensis</i> (100%)	JX291171
24	A3S1-D50	<i>Aspergillus tubingensis</i> (100%)	JX501396
25	A3S1-D107	<i>Aspergillus tubingensis</i> (100%)	JX291170
26	A3S2-12	<i>Aspergillus tubingensis</i> (100%)	JX291172
27	A3S2-D45	<i>Aspergillus tubingensis</i> (100%)	JX291173
28	A3S2-D94	<i>Aspergillus tubingensis</i> (100%)	JX291174
29	A3S3-6	<i>Aspergillus tubingensis</i> (100%)	JX501398
30	A3S3-D40	<i>Aspergillus tubingensis</i> (100%)	JX291175
31	A3S5-2	<i>Aspergillus tubingensis</i> (99%)	JX501403
32	A3S5-32	<i>Aspergillus tubingensis</i> (99%)	JX291195
33	A3S5-D18	<i>Aspergillus tubingensis</i> (99%)	JX291196
34	A3S6-2	<i>Aspergillus tubingensis</i> (100%)	JX501405
35	A3S6-58	<i>Aspergillus tubingensis</i> (99%)	JX501407
36	A3S6-D9	<i>Aspergillus tubingensis</i> (100%)	JX291191
37	A4S1-56	<i>Aspergillus tubingensis</i> (99%)	JX291176
38	A4S2-16	<i>Aspergillus tubingensis</i> (100%)	JX291177
39	A4S3-1	<i>Aspergillus tubingensis</i> (100%)	JX291178
40	A4S4-1	<i>Aspergillus tubingensis</i> (100%)	JX291179
41	A4S5-21	<i>Aspergillus tubingensis</i> (100%)	JX501413
42	A4S5-D2	<i>Aspergillus tubingensis</i> (100%)	JX291180
43	A4S6-28	<i>Aspergillus tubingensis</i> (100%)	JX291181
44	A4S6-D5	<i>Aspergillus tubingensis</i> (100%)	JX291182
45	A1S2-D20	<i>Aspergillus flavus</i> (100%)	JX501354

46	A1S3-D84	<i>Aspergillus flavus</i> (99%)	JX501356
47	A1S4-D2	<i>Aspergillus flavus</i> (100%)	JX501360
48	A1S5-D5	<i>Aspergillus flavus</i> (100%)	JX501363
49	A1S6-3	<i>Aspergillus flavus</i> (100%)	JX501366
50	A1S6-8	<i>Aspergillus flavus</i> (99%)	JX501367
51	A2S1-17	<i>Aspergillus flavus</i> (100%)	JX501369
52	A2S1-D104	<i>Aspergillus flavus</i> (99%)	JX501415
53	A2S2-D29	<i>Aspergillus flavus</i> (100%)	JX501379
54	A2S4-12	<i>Aspergillus flavus</i> (100%)	JX501383
55	A2S4-D25	<i>Aspergillus flavus</i> (99%)	JX501385
56	A2S5-D1	<i>Aspergillus flavus</i> (99%)	JX501391
57	A3S1-D101	<i>Aspergillus flavus</i> (100%)	JX501395
58	A3S3-D3	<i>Aspergillus flavus</i> (100%)	JX501399
59	A3S4-88	<i>Aspergillus flavus</i> (100%)	JX501400
60	A3S4-D2	<i>Aspergillus flavus</i> (99%)	JX501401
61	A3S5-58	<i>Aspergillus flavus</i> (99%)	JX501404
62	A3S6-48	<i>Aspergillus flavus</i> (99%)	JX501406
63	A4S3-12	<i>Aspergillus flavus</i> (99%)	JX501409
64	A4S3-13	<i>Aspergillus flavus</i> (99%)	JX501410
65	A4S6-40	<i>Aspergillus flavus</i> (100%)	JX501414
66	A4S3-D1	<i>Aspergillus tamarisii</i> (99%)	JX501411
67	A1S2-3	<i>Aspergillus aculeatus</i> (100%)	JX291165
68	A1S2-D21	<i>Aspergillus aculeatus</i> (100%)	JX291166
69	A1S3-D48	<i>Aspergillus aculeatus</i> (100%)	JX501355
70	A1S3-D9	<i>Aspergillus aculeatus</i> (100%)	JX501357
71	A1S4-D17	<i>Aspergillus aculeatus</i> (100%)	JX501358
72	A1S4-D18	<i>Aspergillus aculeatus</i> (100%)	JX501359
73	A1S5-D16	<i>Aspergillus aculeatus</i> (99%)	JX501362
74	A1S5-D7	<i>Aspergillus aculeatus</i> (99%)	JX501364
75	A1S6-D11	<i>Aspergillus aculeatus</i> (100%)	JX501368
76	A2S1-4	<i>Aspergillus aculeatus</i> (100%)	JX291167
77	A2S1-D91	<i>Aspergillus aculeatus</i> (100%)	JX501374
78	A2S1-D97	<i>Aspergillus aculeatus</i> (100%)	JX291168
79	A2S1-D98	<i>Aspergillus aculeatus</i> (99%)	JX501375
80	A2S2-D14	<i>Aspergillus aculeatus</i> (100%)	JX501377
81	A2S2-D23	<i>Aspergillus aculeatus</i> (100%)	JX501378
82	A2S3-D3	<i>Aspergillus aculeatus</i> (99%)	JX501381
83	A2S4-D20	<i>Aspergillus aculeatus</i> (99%)	JX501384
84	A2S6-D3	<i>Aspergillus aculeatus</i> (100%)	JX501392
85	A3S1-16	<i>Aspergillus aculeatus</i> (100%)	JX501393
86	A3S1-40	<i>Aspergillus aculeatus</i> (100%)	JX501394
87	A3S1-D57	<i>Aspergillus aculeatus</i> (100%)	JX501397
88	A4S3-D3	<i>Aspergillus aculeatus</i> (100%)	JX501412
89	A1S2-D12	<i>Aspergillus terreus</i> (99%)	JX501352
90	A1S2-D18	<i>Aspergillus terreus</i> (99%)	JX501353
91	A1S4-D36	<i>Aspergillus terreus</i> (100%)	JX501361
92	A2S1-D106	<i>Aspergillus terreus</i> (99%)	JX501372
93	A2S4-D7	<i>Aspergillus terreus</i> (99%)	JX501389
94	A2S4-D50	<i>Aspergillus terreus</i> (99%)	JX501387

95	A3S5-1	<i>Aspergillus terreus</i> (99%)	JX501402
96	A2S3-D7	<i>Aspergillus fumigatus</i> (100%)	JX501382
97	A2S4-D49	<i>Aspergillus fumigatus</i> (99%)	JX501386
98	A2S4-D54	<i>Aspergillus fumigatus</i> (99%)	JX501388
99	A1S3-D53	<i>Aspergillus carbonarius</i> (100%)	JX291202
100	A2S1-D20	<i>Aspergillus ibericus</i> (100%)	JX501373
101	A2S2-D4	<i>Aspergillus ibericus</i> (98%)	JX291203
102	A2S2-D31	<i>Aspergillus sydowii</i> (99%)	KC795922
103	A2S3-D6	<i>Aspergillus sydowii</i> (99%)	KC795923
104	A1S2-D37	<i>Penicillium citrinum</i> (100%)	KC344960
105	A1S3-D70	<i>Penicillium citrinum</i> (99%)	KC344961
106	A1S4-D52	<i>Penicillium citrinum</i> (99%)	KC344962
107	A1S5-D21	<i>Penicillium citrinum</i> (99%)	KC344963
108	A2S6-6	<i>Penicillium citrinum</i> (100%)	KC344966
109	A3S1-D7	<i>Penicillium citrinum</i> (99%)	KC344964
110	A3S6-D1	<i>Penicillium citrinum</i> (100%)	KC344965
111	A1S1-D35	<i>Penicillium oxalicum</i> (100%)	KC344967
112	A1S2-D30	<i>Penicillium oxalicum</i> (100%)	KC344970
113	A1S2-D36	<i>Penicillium oxalicum</i> (100%)	KC344968
114	A1S2-D38	<i>Penicillium oxalicum</i> (100%)	KC344971
115	A1S3-3	<i>Penicillium oxalicum</i> (100%)	KC344969
116	A2S2-D35	<i>Penicillium georgiense</i> (98%)	KC329468
117	A2S4-D60	<i>Penicillium georgiense</i> (98%)	KC329462
118	A3S1-14	<i>Penicillium georgiense</i> (99%)	KC329463
119	A3S1-37	<i>Penicillium georgiense</i> (98%)	KC329464
120	A3S2-6	<i>Penicillium georgiense</i> (98%)	KC329465
121	A3S2-20	<i>Penicillium georgiense</i> (98%)	KC329466
122	A3S3-31	<i>Penicillium georgiense</i> (98%)	KC329467
123	A3S6-47	<i>Penicillium georgiense</i> (98%)	KC329469
124	A1S4-D3	<i>Penicillium chermesinum</i> (97%)	KJ767049
125	A1S4-D37	<i>Penicillium chermesinum</i> (99%)	KJ767050
126	A1S4-D39	<i>Penicillium chermesinum</i> (99%)	KJ767051
127	A1S5-D31	<i>Penicillium siamense</i> (99%)	KJ767052
128	A2S1-D75	<i>Penicillium siamense</i> (99%)	KJ767053
129	A3S2-39	<i>Penicillium siamense</i> (98%)	KJ767054
130	A3S2-40	<i>Penicillium siamense</i> (98%)	KJ767055
131	A2S3-4	<i>Penicillium purpurogenum</i> (100%)	KC344975
132	A3S2-11	<i>Penicillium purpurogenum</i> (100%)	KC344976
133	A3S3-9	<i>Penicillium purpurogenum</i> (100%)	KC344977
134	A3S3-40	<i>Penicillium purpurogenum</i> (100%)	KC344978
135	A4S1-66	<i>Penicillium purpurogenum</i> (100%)	KC344979
136	A4S1-88	<i>Penicillium purpurogenum</i> (99%)	KC344980
137	A4S2-19	<i>Penicillium pimateouiense</i> (100%)	KC344972
138	A4S2-20	<i>Penicillium pimateouiense</i> (100%)	KC344973
139	A4S2-21	<i>Penicillium pimateouiense</i> (99%)	KC344974
140	A2S6-3	<i>Penicillium pinophilum</i> (96%)	KJ767056
141	A2S2-D38	<i>Penicillium minioluteum</i> (94%)	KJ767057
142	A4S6-1	<i>Penicillium ochrochloron</i> (98%)	KJ767058
143	A1S4-D12	<i>Penicillium daleae</i> (99%)	KJ767059

144	A1S4-D42	<i>Penicillium daleae</i> (99%)	KJ767060
145	A1S3-D87	<i>Penicillium rolfsii</i> (99%)	KJ767061
146	A1S1-6	<i>Penicillium verruculosum</i> (99%)	KJ767062
147	A1S5-D11	<i>Penicillium simplicissimum</i> (97%)	KJ767063
148	A1S5-D20	<i>Penicillium simplicissimum</i> (97%)	KJ767064
149	A1S1-D26	<i>Trichoderma harzianum</i> (99%)	KJ767085
150	A1S3-D25	<i>Trichoderma harzianum</i> (99%)	KJ767086
151	A1S4-2	<i>Trichoderma harzianum</i> (100%)	KJ767087
152	A1S5-D1	<i>Trichoderma harzianum</i> (99%)	KJ767088
153	A3S1-D3	<i>Trichoderma longibrachiatum</i> (99%)	KJ767089
154	A4S4-D1	<i>Trichoderma longibrachiatum</i> (99%)	KJ767090
155	A1S1-D75	<i>Trichoderma asperellum</i> (100%)	KJ767091
156	A1S4-D25	<i>Trichoderma ghanense</i> (100%)	KJ767093
157	A1S3-D22	<i>Trichoderma reesei</i> (99%)	KJ767092
158	A1S3-D6	<i>Fusarium oxysporum</i> (99%)	KJ767067
159	A1S3-D44	<i>Fusarium oxysporum</i> (100%)	KJ767068
160	A1S3-D89	<i>Fusarium oxysporum</i> (100%)	KJ774041
161	A2S3-D1	<i>Fusarium oxysporum</i> (99%)	KJ767070
162	A2S4-4	<i>Fusarium oxysporum</i> (100%)	KJ767069
163	A1S1-D12	<i>Fusarium proliferatum</i> (99%)	KJ767071
164	A1S1-D30	<i>Fusarium proliferatum</i> (100%)	KJ767072
165	A2S1-D96	<i>Fusarium proliferatum</i> (100%)	KJ767073
166	A1S3-D91	<i>Hypoxyton investiens</i> (98%)	KJ774043
167	A1S6-D24	<i>Hypoxyton monticulosum</i> (99%)	KJ774046
168	A2S4-D56	<i>Hypoxyton</i> sp. (94%)	KJ774042
169	A2S6-D8	<i>Hypoxyton monticulosum</i> (99%)	KJ774047
170	A2S6-D10	<i>Hypoxyton monticulosum</i> (99%)	KJ774048
171	A2S6-D12	<i>Hypoxyton monticulosum</i> (99%)	KJ774049
172	A3S4-8	<i>Hypoxyton monticulosum</i> (99%)	KJ774050
173	A3S4-D7	<i>Hypoxyton monticulosum</i> (92%)	KJ774051
174	A1S3-D88	<i>Xylaria feejeensis</i> (99%)	KJ767104
175	A1S4-D44	<i>Xylaria feejeensis</i> (99%)	KJ767105
176	A1S4-D47	<i>Xylaria feejeensis</i> (99%)	KJ767106
177	A1S4-D50	<i>Xylaria feejeensis</i> (99%)	KJ767107
178	A2S1-D94	<i>Xylaria feejeensis</i> (99%)	KJ767108
179	A2S4-D43	<i>Xylaria feejeensis</i> (99%)	KJ767109
180	A2S4-D46	<i>Xylaria feejeensis</i> (99%)	KJ767110
181	A3S6-D3	<i>Xylaria psidii</i> (99%)	KJ767111
182	A1S1-D23	<i>Phoma pedeiae</i> (98%)	KJ767074
183	A1S2-D24	<i>Phoma pereupyrena</i> (99%)	KJ767075
184	A1S2-D31	<i>Phoma pereupyrena</i> (99%)	KJ767076
185	A1S4-D16	<i>Phoma herbarum</i> (99%)	KJ767078
186	A1S6-11	<i>Epicoccum sorghi</i> (99%)	KJ767080
187	A1S6-D13	<i>Phoma herbarum</i> (99%)	KJ767079
188	A2S1-D32	<i>Phoma multirostrata</i> (100%)	KJ767077
189	A2S2-D10	<i>Chaetomium globosum</i> (99%)	KJ767122
190	A2S2-D39	<i>Chaetomium globosum</i> (91%)	KJ767123
191	A2S2-D40	<i>Chaetomium globosum</i> (91%)	KJ767124
192	A2S4-D57	<i>Chaetomium globosum</i> (91%)	KJ767125

193	A3S6-D10	<i>Chaetomium nigricolor</i> (99%)	KJ767126
194	A1S1-D9	<i>Paraconiothyrium brasiliense</i> (98%)	KJ767099
195	A1S4-1	<i>Paraconiothyrium brasiliense</i> (97%)	KJ767100
196	A1S4-3	<i>Paraconiothyrium brasiliense</i> (97%)	KJ767101
197	A1S4-4	<i>Paraconiothyrium brasiliense</i> (97%)	KJ767102
198	A2S4-11	<i>Paraconiothyrium brasiliense</i> (100%)	KJ767103
199	A1S2-D2	<i>Curvularia geniculata</i> (97%)	KJ767098
200	A1S2-D16	<i>Curvularia tuberculata</i> (99%)	KJ767096
201	A1S5-D25	<i>Curvularia geniculata</i> (99%)	KJ767097
202	A2S2-1	<i>Curvularia lunata</i> (99%)	KJ767095
203	A1S5-4	<i>Engyodontium album</i> (99%)	KJ767112
204	A1S5-6	<i>Engyodontium album</i> (99%)	KJ767113
205	A1S5-8	<i>Engyodontium album</i> (99%)	KJ767114
206	A1S6-6	<i>Engyodontium album</i> (99%)	KJ767115
207	A1S1-D10	<i>Robillarda sessilis</i> (99%)	KJ767082
208	A1S1-D20	<i>Robillarda sessilis</i> (99%)	KJ767083
209	A1S1-D32	<i>Robillarda sessilis</i> (99%)	KJ767084
210	A2S4-2	<i>Robillarda sessilis</i> (99%)	KJ767081
211	A2S6-D6	<i>Annulohypoxylon nitens</i> (99%)	KJ774044
212	A2S6-D13	<i>Annulohypoxylon nitens</i> (100%)	KJ774045
213	A1S1-1	<i>Cladosporium cladosporioides</i> (100%)	KJ767065
214	A1S5-D28	<i>Cladosporium cladosporioides</i> (100%)	KJ767066
215	A1S4-D8	<i>Humicola fuscoatra</i> (99%)	KJ767116
216	A4S5-D3	<i>Humicola fuscoatra</i> (99%)	KJ767117
217	A1S3-D17	<i>Lasiodiplodia crassispota</i> (99%)	KJ774058
218	A1S3-D20	<i>Lasiodiplodia crassispota</i> (99%)	KJ774059
219	A1S2-D10	<i>Lasiodiplodia theobromae</i> (99%)	KJ774056
220	A3S5-D1	<i>Lasiodiplodia theobromae</i> (100%)	KJ774057
221	A1S5-12	<i>Letendraea helminthicola</i> (100%)	KJ774052
222	A2S3-1	<i>Letendraea helminthicola</i> (96%)	KJ774053
223	A1S2-D13	<i>Microsphaeropsis arundinis</i> (99%)	KJ774054
224	A1S4-D46	<i>Microsphaeropsis arundinis</i> (99%)	KJ774055
225	A1S2-2	<i>Myrothecium inundatum</i> (100%)	KJ767118
226	A2S4-D45	<i>Myrothecium inundatum</i> (100%)	KJ767119
227	A1S1-D38	<i>Nigrospora sphaerica</i> (100%)	KJ767120
228	A2S1-D3	<i>Nigrospora sphaerica</i> (99%)	KJ767121
229	A1S3-1	<i>Pestalotiopsis neglecta</i> (100%)	KJ767128
230	A1S3-6	<i>Pestalotiopsis disseminata</i> (100%)	KJ767129
231	A2S1-D84	<i>Arthrimum phaeospermum</i> (99%)	KJ780759
232	A1S1-D17	<i>Bartalinia pondoensis</i> (99%)	KJ767127
233	A1S6-2	<i>Bipolaris sorokiniana</i> (98%)	KJ767094
234	A2S6-4	<i>Bionectria epichloe</i> (99%)	KJ780769
235	A2S1-D99	<i>Botryosphaeria dothidea</i> (99%)	KJ780760
236	A2S6-14	<i>Camarosporium</i> sp. (100%)	KJ780771
237	A1S5-D23	<i>Ceriporia lacerata</i> (99%)	KJ780757
238	A2S6-11	<i>Coprinellus disseminatus</i> (95%)	KJ780770

239	A2S2-D11	<i>Coprinellus radians</i> (99%)	KJ780763
240	A2S3-5	<i>Coprinopsis cinerea</i> (99%)	KJ780765
241	A2S6-15	<i>Coriopsis polyzona</i> (99%)	KJ780772
242	A2S5-34	<i>Daldinia eschscholzii</i> (99%)	KJ780768
243	A2S4-D6	<i>Emericella similis</i> (99%)	KJ780775
244	A1S2-D48	<i>Graphium penicillioides</i> (99%)	KJ780752
245	A1S5-10	<i>Hansfordia sinuosae</i> (99%)	KJ780756
246	A1S2-D14	<i>Microdochium nivale</i> (96%)	KJ780751
247	A1S3-D34	<i>Montagnulaceae</i> sp. (100%)	KJ780753
248	A1S2-D11	<i>Nectria haematococca</i> (99%)	KJ780750
249	A1S6-D1	<i>Neurospora calospora</i> (98%)	KJ780758
250	A1S6-D28	<i>Paecilomyces variotii</i> (99%)	KJ780746
251	A1S3-D98	<i>Perenniporia tephropora</i> (99%)	KJ780755
252	A2S4-D47	<i>Periconia macrospinoso</i> (95%)	KJ780767
253	A2S2-7	<i>Phaeosphaeriopsis musae</i> (99%)	KJ780762
254	A2S1-D107	<i>Phanerochaete chrysosporium</i> (99%)	KJ780761
255	A2S4-D41	<i>Preussia minima</i> (98%)	KJ780766
256	A1S1-D30	<i>Pseudofusicoccum violaceum</i> (99%)	KJ780748
257	A1S3-D96	<i>Pycnidiophora dispersa</i> (99%)	KJ780754
258	A1S4-D41	<i>Scedosporium apiospermum</i> (99%)	KJ780747
259	A1S2-D6	<i>Valsa brevispora</i> (99%)	KJ780749
260	A3S6-D2	<i>Macrophomina phaseolina</i> (99%)	KJ780774
