

## Diversity of Microfungi in Sandy Beach Soil of Teluk Aling, Pulau Pinang

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**Abstrak:** Sejumlah 82 penculan mikrokulat telah dipencilkan daripada 6 sampel tanah pantai yang dikutip dari pantai Teluk Aling, Pulau Pinang. Mikrokulat daripada tanah tersebut dipencilkan melalui kaedah pemencilan terus, pemencilan piung dan teknik pencairan. Berdasarkan pencirian secara morfologi, tujuh genera mikrokulat telah dikenalpasti iaitu *Fusarium* (42%), *Aspergillus* (24%), *Trichoderma* (13%), *Curvularia* (9%), *Colletotrichum* (6%), *Helminthosporium* (4%) and *Penicillium* (2%). Spesies yang paling kerap dipencilkan adalah *Fusarium solani* diikuti dengan *Fusarium semitectum*, *Aspergillus niger*, *Trichoderma viride*, *Curvularia clavata*, *Curvularia lunata*, *Helminthosporium velutinum*, *Colletotrichum* sp. dan *Penicillium chrysogenum*. Daripada kajian yang dilakukan, didapati pantai berpasir mengandungi takungan pelbagai genera mikrokulat yang memberikan sumbangan yang signifikan terhadap fungsi ekologi ekosistem marin.

**Kata kunci:** Diversiti, Mikrokulat, Tanah Pantai

**Abstract:** A total of 82 isolates of microfungi were isolated from 6 sandy soil samples collected from Teluk Aling beach, Pulau Pinang. The soil microfungi were isolated by using direct isolation, debris isolation and soil dilution techniques. Based on morphological characteristics, seven genera of microfungi were identified namely, *Fusarium* (42%), *Aspergillus* (24%), *Trichoderma* (13%), *Curvularia* (9%), *Colletotrichum* (6%), *Helminthosporium* (4%) and *Penicillium* (2%). The most common species isolated was *Fusarium solani* followed by *Fusarium semitectum*, *Aspergillus niger*, *Trichoderma viride*, *Curvularia clavata*, *Curvularia lunata*, *Helminthosporium velutinum*, *Colletotrichum* sp. and *Penicillium chrysogenum*. From the present study, it appears that the sandy beach contains a microfungi reservoir comprising of a variety of genera which contributes significantly to the ecological functioning of a marine ecosystem.

**Keywords:** Diversity, Microfungi, Beach Soil

## INTRODUCTION

Sandy beaches are located at the junction between water and land which are defined by the sand, wave and tide regimes (Schlacher *et al.* 2008) and usually consist of loose deposits of sand, crushed shells, rocks, gravel and pebbles. The soil of sandy beaches supports a high population of organism such as small invertebrates, bacteria, fungi, yeast, virus, algae and diatoms which can adapt in constantly changing environment. These organisms live between the sand grains and they are involved in the food chain interactions (Larsen & Doggett 1990).

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Microorganisms such as microfungi are significant components of sandy beaches soil and play an important role in decomposition of organic matter in sand and water, as well as in nutrient cycling and degradation of hydrocarbon (Moore-Landecker 1996). Microfungi in sandy soils can be a saprophyte, mutualist or pathogen on marine plants and invertebrates. The saprophytic microfungi play an important role as decomposers of cellulose in the form of washed-up leaves, algae and animal products such as chitin, keratin and calcium carbonate (Kohlmeyer *et al.* 2004). In addition, it may also act as opportunistic pathogens to human (de Hoog *et al.* 2000).

Various factors have been proposed as being encouraging for the survival and dispersion of fungi on sandy beach soils. These include the nature of the beach, tidal phenomena, sewage outlets and season as well as animal and human activities. Water movement, for example, causes erosion, transportation and deposition of beach sediment and redistribution of associated fungi (Mendes *et al.* 1998).

Most of the studies on soil microfungi in sandy beach have been conducted by researchers in Europe such as in Greece (Papadakis *et al.* 1997), Italy (Mancini *et al.* 2005), Spain (Roses Codinachs *et al.* 1988; Larrondo & Calvo 1989) and also in Egypt (Alexandria beach) (Fatma 2003) and Brazil (Gomes *et al.* 2008). In Malaysia, knowledge on soil microfungi in sandy beach is still lacking. Therefore, this study was carried out in order to observe the diversity of microfungi in sandy beach soil samples of Teluk Aling, Teluk Bahang, Pulau Pinang which is located at the north-western tip of Pulau Pinang and is part of the Penang National Park.

## **MATERIALS AND METHODS**

### **Soil Sample**

Six soil samples were collected randomly from Teluk Aling beach, Teluk Bahang (Table 1). The soils were taken by scraping off the surface and subsurface to a depth of 10 cm. Approximately 1.5 kg of soils were collected from each site and put in plastic bags and labeled based on the collection sites.

All the soil samples were air-dried at room temperature ( $27\pm 1^{\circ}\text{C}$ ) for 48 to 72 h. The dried soil samples were sieved through a 0.5 mm sieve to remove stones and plant residues. Sieved soils and debris were then stored separately in sterile paper bags and kept in a refrigerator at  $4^{\circ}\text{C}$ . Plastic bags were not used to store the soil samples as these bags do not allow the soil samples to dry completely and may encourage bacterial growth (Leslie & Summerell 2006).

### **Soil Analysis**

The soil samples were analysed for their texture, pH and moisture. Soil texture was determined using the feel method described by Brady and Weil (2008) and the soil texture was classified based on Biondo and Lee (1997).

**Table 1:** Soil samples and the vegetation.

Code	Collection site
S1	Roots of sea hibiscus ( <i>Hibiscus tiliaceus</i> )
S2	Beach waterline
S3	Beds of beach morning glory ( <i>Ipomoea pes-caprae</i> )
S4	Underneath sea almond tree ( <i>Terminalia catappa</i> )
S5	Among rocks at the waterline
S6	Underneath screwpine tree ( <i>Pandanus odoratissimus</i> )

Soil pH was measured by weighing 30 g of the soil samples in a 100 ml beaker and 75 ml of distilled water was added and mixed well. The mixture was then incubated at room temperature ( $27\pm 1^\circ\text{C}$ ) for 24 h to ensure all substances in the soil sample were diluted. The pH reading was taken by using a pH meter (Mettler Toledo Delta 320, Greifensee, Switzerland) and recorded after 24 h of incubation. The average of the pH reading was then calculated to ensure the accuracy of the results (Head 1980).

For calculation of soil moisture, 10 g of each soil sample was put in a Petri dish and weighted. Then, the soil sample was incubated in an oven at  $105^\circ\text{C}$  for 48 h. After 48 h of incubation, the soil sample was taken out and cooled down at room temperature. The soil sample was weighted again and the moisture content of the soil sample was calculated (Head 1980). The percentage of moisture in the soil samples was calculated based on the following formula:

$$\text{Soil moisture (\%)} = \frac{(\text{weight of petri dish + soil before incubation}) \text{ g} - (\text{weight of petri dish + soil after incubation}) \text{ g}}{(\text{weight of soil sample}) \text{ g}}$$

### Isolation and Identification of Sandy Beach Soil Microfungi

Three methods namely, soil dilution plate, debris isolation and direct isolation techniques with some modifications were used to isolate the microfungi in the soils. The three techniques used were based on the methods recommended and outlined by Bills *et al.* (2004) for isolation of soil saprophytic fungi and the media used was potato dextrose agar (PDA) from Oxoid Ltd. (Basingstoke, Hampshire, England), amended with streptomycin sulphate. The plates were incubated at  $27\pm 1^\circ\text{C}$  for 7 days or until mycelia growth were visible on the plates.

For direct isolation technique, 2 g of the sandy soils was distributed evenly on the medium, incubated at  $27\pm 1^\circ\text{C}$  until visible fungal colonies were observed. Plant debris recovered from the soil samples were cut into small pieces, about 10–15 mm and plated directly on the PDA plates. The mycelia

growing from the plant debris were then transferred onto new PDA plates. For soil dilution technique, 10 g of sandy soils were used for dilution series until  $10^{-4}$  dilution and from each dilution, 1 ml of the soil suspension from each dilution was transferred. Then, the suspension was evenly distributed on PDA plates with a spreader (hockey stick) and incubated at  $27\pm 1^{\circ}\text{C}$ . The fungal colonies grown on the plates were observed after 5–7 days. The number of colonies formed on the media were counted and recorded to calculate the colony forming unit (CFU) of the isolates.

For identification purposes, pure cultures were obtained by using single spore technique. The isolates were then identified using morphological characteristics according to the methods and descriptions of Ellis (1971), Barron (1972), Nelson *et al.* (1983), Barnett and Hunter (2006), The *Fusarium* Laboratory Manual (Leslie & Summerell 2006) and Samson *et al.* (2010).

## RESULTS AND DISCUSSION

The soil texture for the six soil samples collected can be categorised into two types which were sand and loamy sand (Table 2). Both of these soil types gave a gritty feeling and will form crumbled ball when moistened and is regarded as sandy soils (Brady & Weil 2008). The majority of sandy soil contents are sand particles which made up to about 85%–100% for sand and about 70%–90% in loamy sand. The percentage of silt and clay particles in sandy soil is relatively small compared to the sand particles. In sand, there are only about 0%–15% of silt particles and 0%–10% of clay particles. However, the silt and clay contents are relatively higher in loamy sand which contains about 0%–30% of silt particles and 0%–15% of clay particles (Brady & Weil 2008).

The pH value of the sandy beach soil samples was slightly alkaline ranging from 7.63 to 8.62 (Table 2). Soil sample S3 was the most alkaline (pH 8.62) among all the soil samples. Although the optimal pH that favours the growth of different types of fungi varies greatly, majority of fungi seem to grow best at pH 4 to pH 7 (Alexopoulos *et al.* 2002). However, *Fusarium* sp. can tolerate a wide pH range and thus variation in pH does not seem to affect species prevalence and density except possibly in extreme conditions (Mandeeel 2006).

Moisture level of the sandy beach soils was very low with the average of 0.008%–0.258% (Table 2). Soil moisture depended on the availability of water and the retention of water in soils. The low moisture in sandy beach soil is due to the large pore spaces between the soil particles. After heavy rain or irrigation, gravitational water is drawn through the soil. Upon drainage of excess water, water is retained at field capacity in soil pores and this water is known as capillary water (Subba Rao 2001). In sandy beach soil, water is easily drained due to gravitational forces and hence little water is retained among the soil particles. Therefore, the moisture level of sandy beach soil is relatively lower compared to other types of soil.

**Table 2:** Texture, pH and moisture conditions, and microfungi isolates recovered from sandy beach soil samples using soil dilution plate, direct isolation and debris isolation techniques.

Soil sample	Texture	pH	Moisture (%)	Isolation technique		
				Soil dilution	Direct isolation	Debris isolation
S1	Sand	7.63	0.211	<i>A. niger</i> (1) <i>F. solani</i> (1) <i>P. chrysogenum</i> (1)	<i>F. semitectum</i> (1) <i>F. solani</i> (4) <i>T. viride</i> (3)	–
S2	Loamy sand	8.05	0.258	<i>F. semitectum</i> (1) <i>F. solani</i> (1)	<i>A. niger</i> (1) <i>T. viride</i> (3)	–
S3	Loamy sand	8.62	0.030	<i>A. niger</i> (3) <i>F. solani</i> (1)	<i>A. niger</i> (4) <i>F. semitectum</i> (4)	<i>Colletotrichum</i> (5) <i>Curvularia clavata</i> (5) <i>Curvularia lunata</i> (1) <i>F. semitectum</i> (8) <i>F. solani</i> (8) <i>H. velutinum</i> (3)
S4	Loamy sand	8.29	0.008	<i>A. niger</i> (4)	<i>P. chrysogenum</i> (1) <i>T. viride</i> (1)	–
S5	Sand	8.05	0.081	<i>A. niger</i> (1)	<i>A. niger</i> (1) <i>F. solani</i> (2) <i>T. viride</i> (2)	–
S6	Sand	8.30	0.025	<i>A. niger</i> (5)	<i>C. clavata</i> (1) <i>F. semitectum</i> (2) <i>F. solani</i> (1) <i>T. viride</i> (2)	–

A total of 82 isolates of microfungi were recovered from the 6 sandy beach soil samples comprising 34 isolates of *Fusarium*, 20 *Aspergillus*, 11 *Trichoderma*, 7 *Curvularia*, 5 *Colletotrichum*, 3 *Helminthosporium* and 2 *Penicillium* (Table 2). All the genera of microfungi recovered in the present study have been isolated from sandy soil and marine habitat in Portugal (Mendes *et al.* 1998), Italy (Mancini *et al.* 2005), Brazil (Sarquis & Oliveira 1996; Gomes *et al.* 2008) and Egypt (Fatma 2003). Among the factors which could influence the occurrence and diversity of microfungi in sandy beach soils are pH, the extent of salinity, heat from the sunlight and wind (Hyde 1989).

Among the soil samples, S3 had the highest number of microfungi isolated in which 42 isolates were obtained followed by S1 and S6 soil samples (Table 2). Plant debris was abundant in S3 soil samples and only a small quantity of debris was recovered from the other soil samples. Therefore, the debris plating technique was successful for isolating microfungi in soil sample S3 which was taken from the bed of beach morning glory plants.

From the results obtained, it can be seen that the content of organic matter would affect the distribution of sandy beach soil microfungi. The content of organic matter in soils is a potential source of N, P and S for fungal growth. Microbiological decomposition of organic matter is an essential step to release the bound nutrients in organic residues into utilisable form by microfungi (Subba Rao 2001). Thus, the soil microfungi would prefer locations high in organic matter content such as plant roots as their habitat.

From the present study, the highest number of microfungi was recovered from direct isolation technique in which 34 isolates were isolated followed by debris isolation technique with 30 isolates and soil dilution technique with 19 isolates (Table 2). The CFU was unable to perform due to the low number of colonies formed on the plates of soil dilution series. The number of colonies formed were lesser than 30 colonies, and hence, it was insignificant to be considered for counting CFU units. The ideal number for statistically valid enumeration of colonies for CFU was between 30 to 300 colonies (Koch 1994; Foght & Aislabie 2005).

Among the 34 isolates of *Fusarium* recovered from the sandy beach soil samples, 21 isolates were isolated from soil sample S3 by using debris isolation technique. The debris isolation technique recovered the most number of *Fusarium* isolates. According to Leslie and Summerell (2006), recovering isolates from debris usually expands the spectrum of species recovered by including species that are endophytes or live within the plant tissue.

*Fusarium solani* was the most common species isolated from the sandy beach soils followed by *Fusarium semitectum*. *F. solani* is widely distributed in numerous native soils in sub tropical, semi-arid and grassland soils (Burgess & Summerell 1992) and were regularly isolated from different types of soils such as from agricultural soils (Lim & Chew 1970; Latiffah et al. 2007), mangrove soils and forest soils (Latiffah et al. 2010a,b). *F. semitectum* is commonly isolated from soil in tropical, sub-tropical areas and from soils in the Arctic (Kommendahl et al. 1988), deserts (Joffe & Palti 1977), agricultural soils (Latiffah et al. 2007) and most probably exist as soil inhabitants.

The 20 isolates of *Aspergillus* isolated from the soil samples was identified as *A. niger* which was recovered from all the 6 soil samples. It has been reported that the genus *Aspergillus* was one of the most common microfungi present in Mediterranean coast beaches (Larrondo & Calvo 1989), sandy soil of Ipanema Beach, Rio de Janeiro, Brazil (Sarquis & Oliveira 1996) and beach sand samples in Spain (Roses Codinachs et al. 1988).

Although *Trichoderma* is one of the most ubiquitous soil fungi (Barron 1972), only 11 isolates were isolated from 5 soil samples. The isolates were identified as *T. viride*. Similar results were also reported by Fatma (2003) in which the occurrences of *T. viride* was lower in sandy soils of Egypt. In a study by Gomes *et al.* (2008) *Trichoderma* sp. was isolated from two beaches in Brazil during dry and rainy seasons.

Among the total of seven isolates of *Curvularia*, six isolates were recovered by using debris isolation technique and only one isolate was recovered by direct isolation technique (Table 2). Six isolates was identified as *C. clavata* and only one isolate as *C. lunata*. The genus *Curvularia* is most frequently encountered as a parasite or saprophyte of graminaceous hosts. Although reported from soil from time to time, it is seldom recorded in high frequencies and *C. lunata* as well as *C. geniculata* were the most commonly recovered species (Barron 1972).

All the five isolates of *Colletotrichum* sp. were recovered by using debris isolation technique from soil sample S3. *Colletotrichum* is one of the most common plant pathogenic fungi and the fungi survive by colonising plant debris before being incorporated in the soil (Vizvary & Warren 1974). Some species of *Colletotrichum* have been reported to produce sclerotia and would survive in the soils for a long time (Farley 1972). Occurrences of *Colletotrichum* in sandy soils were reported by Gomes *et al.* (2008) in two beaches of Bairro Nova and Casa Caiada in Brazil.

The three isolates of *Helminthosporium* were identified as *H. velutinum* isolated from soil sample S3 by using the debris isolation technique. *Helminthosporium* was among the microfungi recovered from sandy soil samples of Alexandria beach in Egypt (Fatma 2003). According to Ellis (1971), *H. velutinum* was very common on dead stems of herbaceous plants and twigs and branches of many different kinds of trees.

In this study, two isolates of *Penicillium* were recovered from the sandy soil samples and the isolates were identified as *P. chrysogenum*. The results of the present study contrasted with studies by Larrondo and Calvo (1989), Sarquis and Oliveira (1996) and Fatma (2003). Larrondo and Calvo (1989) who carried out a study on the fungal diversity of Mediterranean coast beaches, managed to isolate 24 different species of *Penicillium*. Fatma (2003) reported that *Penicillium* was the most frequent species isolated from sandy soil samples of Alexandria beach in Egypt. *Penicillium* was also one of the most common fungal genera isolated from Ipanema beach in Brazil (Sarquis & Oliveira 1996).

In general, the occurrence and diversity of microfungi in Teluk Aling sandy beach soils were similar with other study in different parts of the world. The differences in species composition may be due to different sampling strategies, different isolation techniques, salinity of the seawater, temperature and nutrient status as indicated by Jones (2000).

Although many species of microfungi isolated from different types of soils are saprophytic, some species are biotrophic mutualists and parasites of plants, animals and other fungi (Bills *et al.* 2004). A small number of these microfungi

may act as incidental or transient invaders in which the spores or propagules are introduced accidentally and remain dormant (Bills *et al.* 2004).

As a conclusion, a total of 82 isolates of microfungi were recovered from the sandy soil samples at Teluk Aling beach. Seven microfungi genera had been successfully recovered and identified namely, *Fusarium* (42%), *Aspergillus* (24%), *Trichoderma* (13%), *Curvularia* (9%), *Colletotrichum* (6%), *Helminthosporium* (4%) and *Penicillium* (2%). The most common species isolated was *F. solani* followed by *F. semitectum*, *A. niger*, *T. viride*, *C. clavata*, *C. lunata*, *H. velutinum*, *Colletotrichum* sp. and *P. chrysogenum*. Based on the present study, it appears that sandy beach soils which is part of the marine ecosystem contain a variety of microfungi reservoir which contribute significantly to ecological functioning of the ecosystem.

## ACKNOWLEDGEMENT

The authors would like to thank Department of Wild Life and National Parks (PERHILITAN) and Penang National Park authorities for giving us the permission to collect the sandy beach soil samples from Teluk Aling.

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