# Isolation And Characterization of Fungi From Mangrove Soils

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## Abstract

In this study, the four soil samples were collected from Nga Moe Yeik creek in North Dagon Township and Lein Kone creek in Shwe Pyi Thar Township, Yangon Region. The sixteen mangrove fungi were isolated from the collected samples on five different media by the serial dilution method. Nine mangrove soil fungal strains were isolated from Nga Moe Yeik creek and seven mangrove soil fungal strains were isolated from Lein Kone creek. The morphological and microscopical characters of all fungal strains were carried out at Microbiology Laboratory, Department of Botany, Dagon University. The colony appearances of isolated fungal strains were irregular, circular, filamentous and punctiform. The colony margins of isolated strains were entire, filamentous, undulate, curled and lobate. The elevations of isolated strains were raised, flat, convex and umbonate. The surface and reverse colors of isolated fungal strains were white, yellow, whitish orange, orange, greyish white, brownish grey, blue-green. greenish yellow, greenish black and black. The colony characters and spore types of isolated fungi were very closed to *Penicillium*, *Cladosporium* and *Paecilomyces* species. Strains EP-2, EP-4, EP-5, EP-9, EP-15 and EP-16 were identified as *Penicillium* species, strains EP-3, EP-6, EP-7, EP-8, EP-10 and EP-11 as *Cladosporium* species, and EP-13 and EP-14 as *Paecilomyces* species. These isolated strains would be possessed antimicrobial activity on some pathogenic organisms, so that this research should be continued to investigate bioactivity of all strains.

***Keywords*:** *Cladosporium* sp., *Paecilomyces* sp. and *Penicillium* sp., Mangrove soil

## Introduction

There are many different types of microorganisms in mangrove soils. Among them, mangrove fungi are well known for the productivity and maintenance of ecosystem. These fungi play an important role in nutritive cycle and degradation of organic wastes by secreting extracellular carbohydrates such as cellulase, xylanase, pectinase, amylase, etc. In the mangrove area, there are lower fungi and higher fungi.These fungi are also called “manglicolous fungi” in which both of marine as well as terrestrial fungi are included. tolerant forest ecosystem existed in the intertidal zones of sheltered estuaries, tidal creeks, backwaters, lagoons, marshes and mudflats (Kohlmeyer, 1969).

*Penicillium* species are potential producers of natural pigments. Pigments in fungi are not essential for their growth and development and therefore, classified as secondary metabolites with wide application. The secondary products were derived from primary metabolism and have been used as anti-fungal and anti-bacterial (Omura, 1992).

*Paecilomyces* species have been used as Chinese traditional medicine to treat impotence, sedation, analgesia, backache, cancer, memory loss, and also as a tonic to nourish the lungs and kidneys. *Paecilomyces* species are a source of bioactive natural products. A total of 148 active metabolites has been produced by different *Paecilomyces* species and they can be used for drug or agrochemical development (Li *et al*., 2020).

*Cladosporium* species could be isolated from the soil, mangrove and organic matters. Under the microscope, vegetation hyphae, conidiophore and conidia were equally pigmented. Vegetative hyphae were erect, straight, branched or unbranched.

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Some conidia were produced in branched acropetal chains. Most of conidia were smooth, verrucose or echinulate and contained 1 to 4 celled (Berisch et al., 2012). Their hyphae were filamentous and septate. The present study makes an attempt to survey fungal strains in the collected soils from mangrove area of Nga Moe Yeik creek and Lein Kone creek, Yangon Region. The objectives of this study are to isolate fungal strains from the collected soil samples, to investigate the morphological and microscopical characters of isolated fungal strains, to identify the possible genera of isolated fungal strains.

## Materials AND Methods

### Collection of Soil Samples

The two different soil samples were collected from mangrove areas of Nga Moe Yeik creek in North Dagon Township and the next two different soils samples were also collected from mangrove area of Lein Kone creek in Shwe Pyi Thar, Yangon Region (Figure 1). The soil samples (10 g for each sample) were taken between twelve to twenty-four inches depth and put them into the clean plastic bags. The pH of each sample was measured in Figure (2). At the sampling time, the locations of particular points of soil were record by using the Global Positioning System (GPS) Table (1).

Figure (1) Nga Moe Yeik creek and Lein Kone creek


Figure (2) pH value of soil sample

Table (1) Location of the collected soil sample.

| Collection area | Location | | Depth |
|---|---|---|---|
| Nga Moe Yeik creek | N 16º 53′ 30″ | E 96º 09′ 52″ | twelve to twenty-four inches depth |
| Lein Kone | N 17º 00′ 04″ | E 96º 02′ 58″ | twelve to twenty-four inches depth |

### Isolation of Mangrove Soil Fungi

Serial dilutions of soil, plating and streaking techniques described by Salle, 1948 and Collins, 1965 were used for the isolation of microbes.

An appropriate amount (1 g) of soil was introduced into a conical flask containing 99 ml of distilled water to make a soil-water dilution ratio of 1:100. The flask was then shaken for about 30 minutes in order to make the soil particles free from each other. This solution was then serially diluted into $10^{-1}$ to $10^{-8}$ dilution in separate test tubes. 1 ml each of the above dilutions was separately transferred into sterile petridishes under aseptic condition. A sterile pipette was used for each transfer. 0.5 ml of $10^{-8}$ diluted tube was added on each agar medium, then the plate was gently

shaken to get the uniform solution on the medium. These plates were incubated at room temperature for 2 weeks.

**Composition of culture media** (Atlase, 1993)

(1) Nutrient Agar Medium (NA)

| | |
|---|---|
| Nutrient Agar | = 3.5 g |
| Distilled Water | = 100 ml |
| Agar | = 1.0 g |

(2) Potato Dextore Agar Medium (PDA)

| | |
|---|---|
| Potato Dextore Agar Medium | = 3.9 g |
| Distilled Water | = 100 ml |
| Agar | = 1.0 g |

(3) Sucrose/Yeast Extract Medium (SY)

| | |
|---|---|
| Sucrose | = 1.0 g |
| Yeast | = 0.3 g |
| Distilled Water | = 100 ml |
| Agar | = 2.5 g |

(4) Glucose/Yeast Extract Medium (GY)

| | |
|---|---|
| Glucose | = 1.0 g |
| Yeast | = 0.3 g |
| Distilled Water | = 100 ml |
| Agar | = 2.5 g |

(5) Lactose/Yeast Extract Medium (LY)

| | |
|---|---|
| Lactose | = 1.0 g |
| Yeast | = 0.3 g |
| Distilled Water | = 100 ml |
| Agar | = 2.5 g |

**Morphological Characters of Isolated Fungal Strains**

The morphological and colonial characters such as colony appearance, margin, surface and reverse colours, and elevation of all isolated strains were recorded as revealed in the reference of Dubey and Maheswari, 2014.

**Microscopical Characters of Isolated Fungi**

The microscopical characters of all isolated strains (EP-1 to EP-16) were carried out under light microscope with high magnification at Department of Botany, Dagon University. The main characters of hyphae, mycelium, sporangiophores, spore, color formation on upper as well as lower surface were comparatively studied. These are compared to those of fungi with available literatures such as Barnett, 1969.

## Results

**Isolation of Fungi from Soil Samples**

In the present work, soil strains were isolated from the four soil samples on five different media. The pH results of the soil samples and the numbers of soil isolates were mentioned in Table (2). Nine fungal strains were isolated from the two soil samples of Nga Moe Yeik creek and seven fungal strains were isolated from the two soil samples Lein Kone creek. All sixteen fungal strains were maintained into the pure culture. These strains were given as temporary names EP-1 to EP-16.

Table (2) Different media and soil samples

| No. | Culture medium | Soil sample from Nga Moe Yeik (1ft) (pH 7.5) | Soil sample from Nga Moe Yeik (2ft) (pH 7.5) | Soil sample From Lein Kone (1ft) (pH 8) | Soil sample From Lein Kone (2ft) (pH 8) |
|---|---|---|---|---|---|
| 1. | Nutrient Agar | - | 1 | - | - |
| 2. | Potato Dextore Agar | - | 1 | - | - |
| 3. | Sucrose/Yeast Extract Medium | 3 | 1 | 2 | 1 |
| 4. | Glucose/Yeast Extract Medium | 1 | 1 | 1 | 1 |
| 5. | Lactose/Yeast Extract Medium | 1 | - | 1 | 1 |

**Morphological and Microscopical Characters of Isolated Fungi**

In morphological characters, the colony appearance of isolated fungal strains EP-1, EP-2, EP-3, EP-9, EP-10, EP-13, EP-15 and EP-16 were irregular, strains EP-4, EP-6, EP-8, EP-11, EP-12 and EP-14 were circular, strain EP-7 was filamentaus and strain EP-5 was puntiform Fig. 3 to 16.

The margin of isolated fungal strains EP-2, EP-4, EP-6, EP-8 and EP-10 were entire, strains EP-1, EP-3, EP-7, EP-11 and EP-12 were filamentous, strains EP-5, EP-9 and EP-13 were undulate, strains EP-14 and EP-16 were curled and strain EP 15 was lobate Fig. 3 to 16.

The elevation of isolated fungal strains EP-2, EP-3, EP-6, EP-8, EP-9, EP-10, EP-12, EP-13 and EP-16 were raised, strains EP-7, EP-14 and EP-15 were flat, strains EP-4, EP-5 and EP-11 were convex and strain EP 1 was umbonate Figure (3) to (16).

The surface and reverse colors of isolated fungal strains EP-1, EP-2, EP-4, EP-9 and EP-13 were white and pale yellow. Strains EP-6 and EP-7 were greenish black and black, and strain EP-3 were greyish white and whitish blue green and strain EP-14 were greyish white and whitish pale yellow. The colors of strain EP-5 were whitish orange and whitish pale yellow, and strain EP-8 was blue green. Strain EP-10 was greenish yellow, and both colors of strain EP-16 were greyish white and whitish grey. The colors of strain EP-11 were brownish grey and brownish black; strain EP-12 were white and whitish green and the colors of strain EP-15 were whitish orange and orange Fig. 3 to 16.

**Microscopical Characters of Isolated Fungi**

The microscopical characters of isolated mangrove fungi are the same to the genera of *Penicillium, Cladosporium* and *Paecilomyces.* These strains were identified as strains EP-2, EP-4, EP-5, EP-9, EP-15 and EP-16 as *Penicillium* species, strains EP-3, EP-6, EP-7, EP-8, EP-10 and EP-11 as *Cladosporium* species, strains EP-13 and EP-14 as *Paecilomyces* species. Strains EP-1 and EP-12 were being unable to identify their genus level. So, they were assumed as unidentified isolates Fig. 3 to 16.

**Scientific classification**

| ***Penicillium*** | | | ***Paecilomyces*** | | |
|---|---|---|---|---|---|
| Kingdom | : | Fungi | Kingdom | : | Fungi |
| Division | : | Ascomycota | Division | : | Ascomycota |
| Class | : | Eurotiomycetes | Class | : | Eurotiomycetes |
| Order | : | Eurotiales | Order | : | Eurotiales |
| Family | : | Trichocomaceae | Family | : | Trichocomaceae |
| Genus | : | ***Penicillium*** | Genus | : | ***Paecilomyces*** |

**Morphological and Microscopical Characters of All Fungal Strains**

***Cladosporium***

| | | |
|---|---|---|
| Kingdom | : | Fungi |
| Division | : | Ascomycota |
| Class | : | Dothideomycetes |
| Order | : | Capnodiales |
| Family | : | Davidiellaceae |
| Genus | : | ***Cladosporium*** |

The hyphae of strain EP-1 have septate with long cells and weakly branched. Conidiophores long, bearing three phialides; each phialide has one conidium. It was unidentified as shown in Figure (3).

Conidiophores arising from the hyphae in synnemata branches near the apex, ending in a group of phialides; conidia hyaline, 1- celled, mostly ovoid, in basipetal chains. These strains EP-2, EP-4, EP-5, EP-9, EP-15 and EP-16 may be identified as *Penicillium* species as shown in Figure (4, 5, 6, 7, 8, 9).

Conidiophores long, branched variously near the apex, clustered; conidia light, 1 or 2 celled, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon – shaped; often in simple or branched acropetalous chains; saprophytic. These strains EP-3, EP-6, EP-7, EP-8, EP-10 and EP-11 may be identified as *Cladosporium* species as shown in Figure (10, 11, 12, 13, 14, 15).

The hyphae of strain EP-12 were septate, weakly branched and dark. Cylindrical branched hyphae were also present. Conidia were not formed. It was not identified as shown in Figure (16).

Conidiophores and branches more divergent than in *Penicillium*, conidia in basipetal chains, 1-celled, ovoid, hyaline; saprophytic. These strains EP-13 and EP-14 may be identified as *Paecilomyces* species shown in Figure (17, 18).

Figure (3) Morphological and microscopical characters of strain EP -1

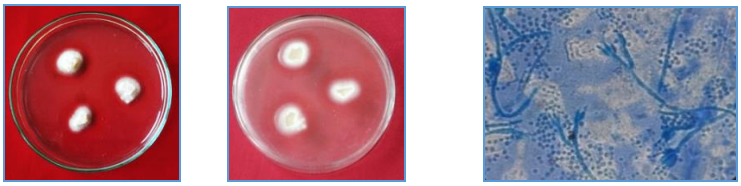

Figure (4) Morphological and microscopical characters of strain EP -2 (*Penicillium* sp.)

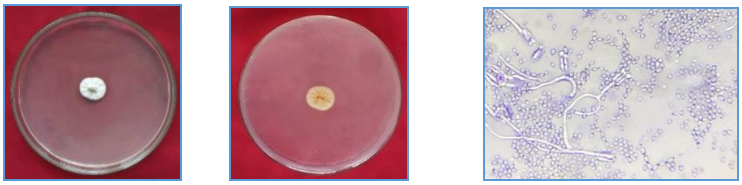

Figure (5) Morphological and microscopical characters of strain EP -4 (*Penicillium* sp.)

Figure (6) Morphological and microscopical characters of strain EP -5 (*Penicillium* sp.)

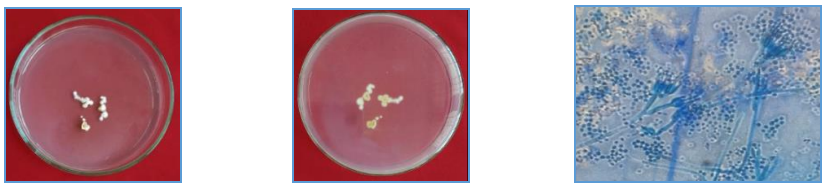

Figure (7) Morphological and microscopical characters of strain EP -9 (*Penicillium* sp.)

Figure (8) Morphological and microscopical characters of strain EP -15 (*Penicillium* sp.)

Figure (9) Morphological and microscopical characters of strain EP -16 (*Penicillium* sp.)

Figure (10) Morphological and microscopical characters of strain EP -3 (*Cladosporium* sp.)

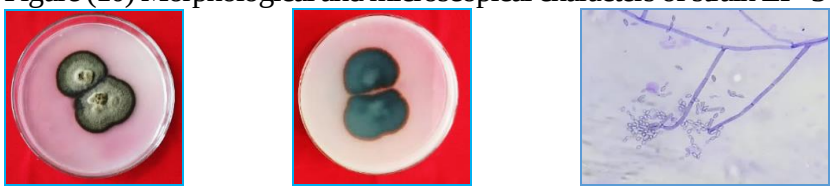

Figure (11) Morphological and microscopical characters of strain EP -6 (*Cladosporium* sp.)

Figure (12) Morphological and microscopical characters of strain EP -7 (*Cladosporium* sp.)

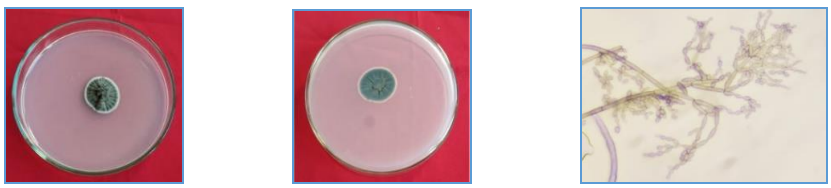

Figure (13) Morphological and microscopical characters of strain EP -8 (*Cladosporium* sp.)

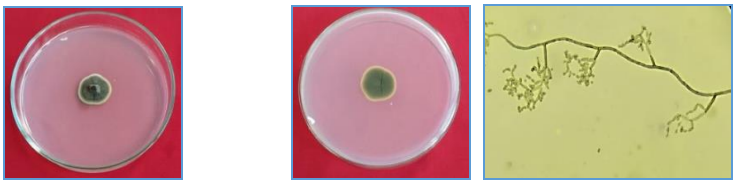

Figure (14) Morphological and microscopical characters of strain EP -10 (*Cladosporium* sp.)

Figure (15) Morphological and microscopical characters of strain EP -11 (*Cladosporium* sp.)

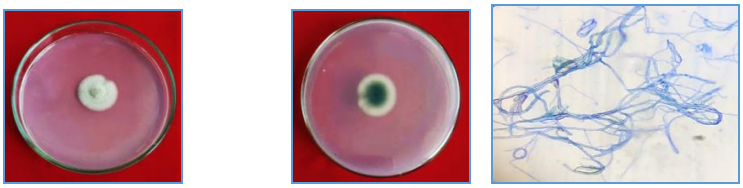

Figure (16) Morphological and microscopical characters of strain EP -12

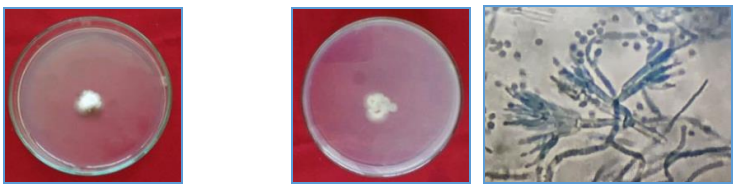

Figure (17) Morphological and microscopical characters of strain EP -13 (*Paecilomyces* sp.)

Figure (18) Morphological and microscopical characters of strain EP -14 (*Paecilomyces* sp.)

## Discussion and Conclusion

In this study, nine mangroves soil fungal strains were isolated from the soil samples of Nga Moe Yeik creek and seven strains were isolated from the soil samples of Lein Kone creek. The different morphological and microscopic characters of all isolated fungi were presented. The different types of colony appearance of soil isolates were circular, irregular, filamentous, and punctiform. The margins of isolated fungi were also various forms such as entire, undulate, curled and lobate. The elevation of isolated fungal colonies was found to be flat, raised, convex and umbonate. These results were in agreement with Dubey and Maheswari (2014).

In this study, the surface colors of isolated colonies were brown, blackish brown and grey. The texture was floccose, often becoming powdery due to the production of abundant conidia. The reverse colonies of the isolated colonies were brown, pale brown, pale yellow green. These results were in agreement with those stated by Davise, 1995.

In the present research among the sixteen isolated strains, the colony, conidiophores and conidia characteristics of strains EP-3, EP-6, EP-7, EP-8, EP-10

and EP-11 were very close to those of *Cladosporium* genus. Therefore, these strains were identified as possible genus *Cladosporium.*

Some conidia were produced in branched acropetal chains. Most of conidia were smooth, verrucose or echinulate and contained 1 to 4 celled. These characteristics are in accordance with those reported by Berisch et al.(2012). They also reported that *Cladosporium* spp. could be isolated from the soil, mangrove and organic matters.

In the study, the fungal colonies of EP-2, EP-4, EP-5, EP-9, EP-15 and EP-16 were found to be white at 24 hours. But they gradually turned into gray, greyish-green, yellow or pinkish. Under the microscope, their hyphae were filamentous and septate. Mycelium was well developed and found profusely branched. Chains of 1-celled conidia were produced in basipetal position from phialide (nonindigenous cell). These phialides formed like a brush (Visagie *et al*., 2014). These characters were very similar to those observed in EP-2, EP-4, EP-5, EP-9, EP-15 and EP-16. These strains were identified as possible genus *Penicillium*.

Among the 16 isolates, EP-13 and EP-14 showed white and whitish grey color and reverse colony color was pale yellow. Upper surface was powdery in texture and fast growing. Phialides were swollen at the base and gradually tapering into a long and slender neck. Conidia were single-celled; hyaline. Their character was partially similar to those discovered by Chen *et al*. (2010). They reported that paecilomyces was a common filamentous fungus usually found in soil and plant waste decay.

In this study, strains EP-13 and EP-14 were identified as possible genus *Paecilomyces* isolated from mangrove soil. Borba *et al*. (2015) reported that the genus *Paecilomyces* has hyaline to yellowish septate hyphae, often with smooth walls and verticillated or irregularly branched conidiophores, and phialides with a wide base and an elongated neck. The conidia are unicellular; hyaline, in chain and the youngest conidium is at the basal end. These characters were similar to those of strains EP-13 and EP-14. It is hoped that these isolated strains would be possessed antimicrobial activity on some pathogenic organisms, so that this research should be continued to investigate bioactivity of all strains as a further research work.

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