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Macroscopic And Microscopic Approaches For Identification Of Fungi From Plant Soil Of Cameron Highlands

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Abstract

Seven cultures of fungi were isolated from vegetable soils in Cameroon Highlands. In this research project two techniques were used for the identification purpose- which are macroscopic and microscopic morphology approaches to identify the genus of the fungi. Macroscopic identification based on the characteristic of the fungus grown on the PDA plate and the microscopic identification was based on the spore and hyphae morphology using lactophenol blue dye. Four genus that have been successfully identified using this approach were *Aspergillus*, *Fusarium*, *Rhizopus* and *Gliocladium*.

INTRODUCTION

About more than 100 000 species of fungi have been described by taxonomists but the global biodiversity of fungi is not fully understood [1]. Fungi are a group of microorganisms that are classified within their own kingdom, the Eumycota (often called kingdom Fungi). Fungi are more like animals than they are like plants for one thing, fungi cannot synthesize their own food like plants do, but instead they eat other organisms as animals do [2]. There are some species of fungi that are single-celled organisms that exist individually, such as yeast. Yeast cells look round or oval under a microscope. There are other kinds of fungi that are known as multi-cellular organisms, such as molds and mushroom. Molds are described as thread-like filamentous microscopic structures called hyphae, which extend at their tips. This apical growth form is in contrast with other filamentous organisms, like filamentous green algae, which grows by intercalary growth. An assemblage of intertwined and interconnected hyphae is called a mycelium. Typically, hyphae will germinate from the spore and grows radially, to form a circular growth of mycelium [3-5].

Fungal mycelia grown on solid agar media in laboratory petri dishes are usually referred to as colonies, with many species exhibiting characteristic macroscopic growth morphologies and colors due to spores or pigmentation. Based on morphological methods, the identification of fungi species requires adequate growth time for evaluation of colony characteristics and microscopic features. A culture time of five days or more is generally required for the development of anamorphic forms of fungi. Specialized fungal structures such as hyphae, fruiting bodies, phialide and conidia were observable in microscopic approach which helps the identification of the species [6].

Reproductive particles of fungi are called spores. They differ in size, shapes and color among species. Each spore that germinates can give rise to a new mold growth, which in turn can produce millions of spores. There are many types of hyphae such as aseptate hyphae, septate hyphae, coenocytic hyphae, skeletal hyphae and many more.

Microscopic examination can be a very helpful approach for the genus identification of the fungi. Thus, in this study, macroscopic and microscopic morphology approaches were being used for the genus identification of fungi isolated from the soil in Cameron Highlands.

MATERIALS AND METHODOLOGY

Fungi Culture

Seven unknown cultures of fungi were used from the Universiti Industri Selangor (UNISEL) culture collections which have been isolated from Cameron Highlands' vegetable soil. The cultures have been isolated from different location in Cameron Highlands (Table 1).

Table 1: Cultures of unknown fungi

Culture	Original location
1	Spinach plant soil
2	Tomato plant Soil
3	Carnation plant Soil
4	Strawberry valley plantation soil
5	Brinjal plant Soil
6	Bharat Tea plant Soil
7	Raju Hill Strawberry plantation soil

Reagents and Chemicals

i) Culture Medium

Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB)

ii) Microscopic observation

Lactophenol Blue

iii) Streaking Method

Streaking a plate is one of the most important basic steps in microbiology. Streaking was done in biohazard safety cabinet. A sterile inoculation loop was used to streak the fungus over the surface of the Potato Dextrose Agar (PDA). The loop has been allowed to glide over the surface of the medium. The handle was being hold at the balance point, sweeping movements, as the agar surface can be easily damaged and torn. The lid of the petri plate must be as close to the base as possible to reduce aerial contamination caused by other microorganisms such as bacteria. After inoculation, the petri plate was sealed with parafilm and incubated at room temperature for four to five days. The streaking method was repeated until the pure culture of each culture was obtained. Pure culture is important for the identification of fungi using macroscopic and microscopic technique and molecular approach.

Stock Culture

The stock culture was prepared using two different methods known as glycerol stock in universal bottles and Potato Dextrose Agar (PDA) slants in bijou bottles. Stock culture is important to make sure that the cultures are not contaminated and for future studies.

i) **Glycerol Stock Preparation**

1 ml of glycerol and 9 ml of distilled water were added to each universal bottle. Then, the universal bottles were autoclaved at 121 °C for 15minutes. In biohazard safety cabinet, the pure cultures were being cut into cubes using a sterile penknife and transferred into the universal bottles. Each culture required at least two bottles of glycerol stock. The universal bottles were then labelled and stored at -20 °C.

ii) **Potato Dextrose Agar (PDA) Slants**

The bijou bottles without caps were placed in a rack. PDA medium has been prepared and a pipette was used to transfer about 15 ml of PDA medium to each bijou bottle. The bijou bottles were sterilized at 121 °C for 15 minutes with caps loosely on. The rack was tilted onto a thick book or other solid surface so that the medium in the bijou bottles were slanted while the bijou bottles were still hot. The medium was allowed to harden in that position. The caps were tightened after the medium cooled. The bijou bottles were ready to be use. In biohazard safety cabinet, a loopful of fungus from the pure culture plate was taken and streaked on the surface of PDA slant.

iii) Staining Method

The most basic reason that cells were stained is that it can enhance visualization of the cell or certain cellular components under a microscope. For fungal staining, three to seven days old cultures of fungus were used. The observation slide was prepared by dropping a drop of lactophenol blue dye and an inoculating loop was used to take a small amount of the fungus and mixed it well with the dye. The slide was then covered with cover slide and was being examined under bright field microscope. The color, shape, appearance and arrangement of the fungus structures were documented. Photographs of the structures were captured using digital camera (Olympus SP-350) for identification purposes.

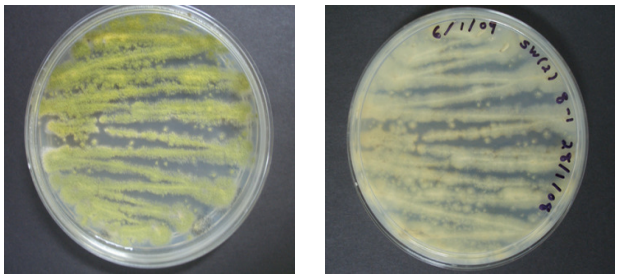
RESULTS AND DISCUSSION

Macroscopic and Microscopic Morphology Observation

In this experiment, PDA medium was used for macroscopic morphology observation. The macroscopic morphology observation was based on the pure culture plate of the fungi sample which was incubated at room temperature (27 °C) for three to four days. The growth rate, colour of the colony and texture of the culture were the most remarkable features during this observation. For microscopic observation, a very small amount of the fungus are stained with lactophenol blue for better cells and cell components visualization under a microscope. From the microscopic observation, the color, shape, appearance and arrangement of the fungus structures were identified.

Culture 1

Culture 1 has a rapid growth rate. The colonies are yellow at first but quickly turn bright to dark yellow-green with age. The texture is powdery and granular. The reverse side of the culture shows pale yellow in colour (Figure 1). The microscopic observation shows the presence of hyaline hyphae, conidiophores, phialides and conidia in culture 1 (Figure 2). The conidiophores were originated from the basal foot cell located on the supporting hyphae and terminated in a vesicle apex. Vesical is a typical formation for the genus *Aspergillus* [7]. Phialides are hyaline, flask shaped, biseriate and attached to the vesicle via a supporting cell metula. Conidia, which have a round shape, are located over the phialides [8].



(a) **(b)**

Figure 1: Macroscopic view of culture 1 (a) Front view (b) Back view

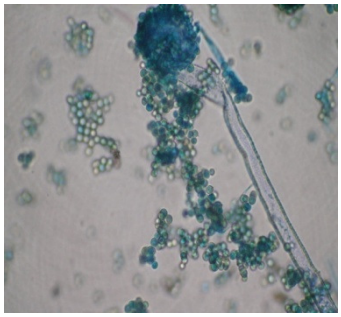


Figure 2: Microscopic view of culture 1 (400x magnification)

Culture 2

Culture 2 has a rapid growth rate and colonies on potato dextrose agar (PDA) at room temperature of 25 °C are initially white, quickly turns to black with conidial production. The texture is powdery and granular. Reverse is pale yellow and growth may produce radial fissures in the agar (Figure 3). Its hyphae appeared to be hyaline. Conidial heads were radiated initially, splitting into columns at maturity. The species is biseriate (vesicles produces sterile cells known as metulae that support the conidiogenous phialides). Vesical is the typical formation for the genus *Aspergillus* [7]. Conidiophores are long, smooth, and hyaline, becomes darker at the apex and terminating in a globose vesicle (Figure 4). Metulae and phialides cover the entire vesicle. Conidia are brown to black, very rough and globose [8].

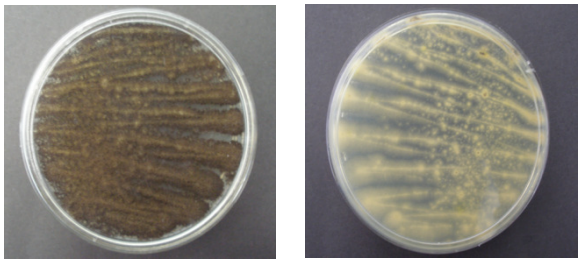


Figure 3: Macroscopic view of culture (a) Front view (b) Back view

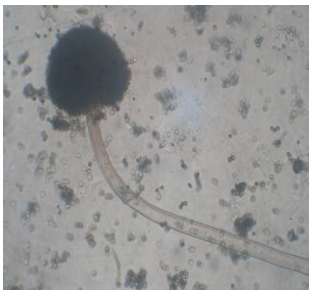
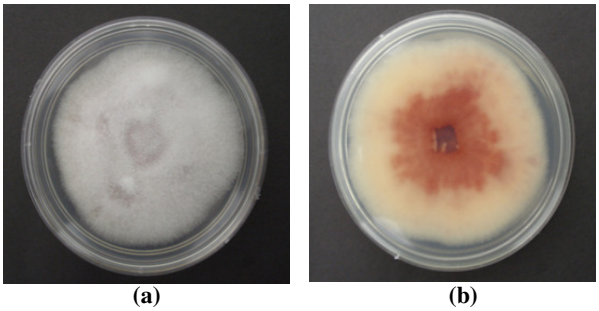


Figure 4: Macroscopic view of culture 2(400X magnification)

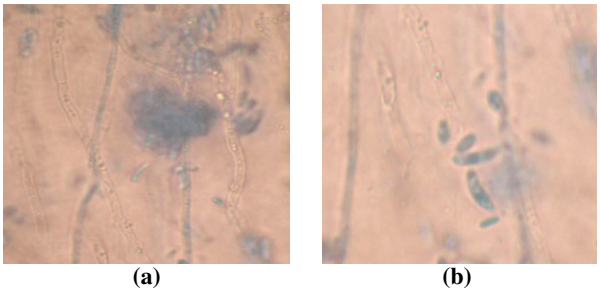
Culture 3

Culture 3 colonies are fast growing and the texture is cottony. Colonies that are initially white, becomes pink at maturity. Reverse is dark pink (Figure 5). Hyphae are hyaline and conidiophores are simple and not branched. Macroconidia were produced abundantly, slightly sickle-shaped, thin-walled, with an attenuated apical cell and a foot-shaped basal cell (Figure 6). Microconidia are abundant, mostly non-septate, ellipsoidal to cylindrical, slightly curved or straight, occurring in false heads (a collection of conidia at the tip of the phialide) from short monophialides [9]. Chlamydoconidia were presented and often abundant, occurred singly and in pairs. Colony and culture characteristics are similar to *Fusarium* sp [10].



(a) **(b)**

Figure 5: Macroscopic view of culture 3 (a) Front view (b) Back view

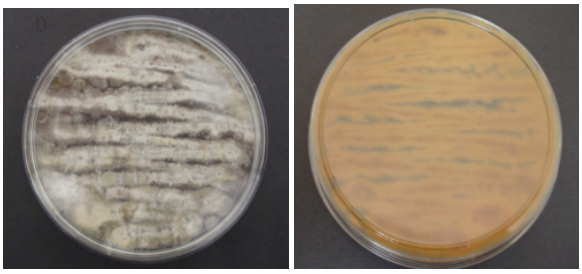


(a) **(b)**

Figure 6: Microscopic view of culture (400X magnification): (a) Hyaline hyphae (b) Macroconidia

Culture 4

Culture 4 has a rapid growth rate. The texture is granular. Colonies are very fast growing at 25 °C, white cottony initially becomes brownish grey and reverse is pale orange (Figure 7). Nonseptate hyphae, sporangiophores, rhizoids (root-like hyphae), sporangia, and sporangiospores were visualized. Sporangiophores are brown in colour and usually unbranched (Figure 8). They can be in solitary or form clusters. Rhizoids are located at the point where the stolons and sporangiophores meet. Sporangia are located at the tip of the sporangiophores. They are surrounded with flattened bases [11]. Sporangiospores are unicellular, round to ovoid in shape, hyaline to brown in color, and smooth or striated in texture. The macroscopic and microscopic characteristics shows that sample 4 is a *Rhizopus* sp. based on the microscopic morphological features of the *Rhizopus* sp. [7]. Culture 4 isolated from strawberry plant soil and common black bread mold, *Rhizopus* sp. is one of the most common species that destroy strawberries and other fruits that are high in sugar content [12].



(a) (b)

Figure 7: Macroscopic view of culture 4 (a) Front view (b) Back view

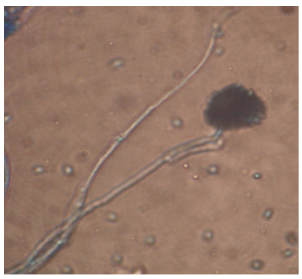


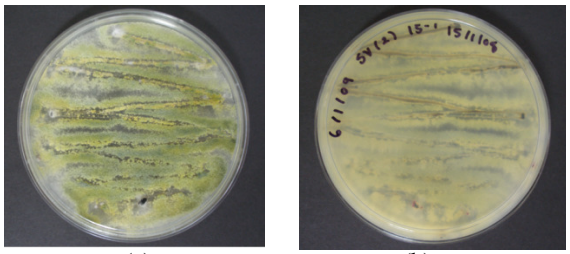
Figure 8: Microscopic view of culture 4 (400X magnification)

Culture 5

Culture 5 has a rapid growth rate and the texture is granular and powdery. The colonies are white at first but quickly turns to smoky gray-green with age [10]. Reverse is pale yellow (Figure 9). Hyphae are septate and hyaline. Conidiophores appeared and it is hyaline and not branched (Figure 10). Phialides are hyaline and over the phialides are the conidia. This species is uniseriate with closely compacted phialides occurring only on the upper portion of the vesicle [8]. Conidia are round, ellipsoidal to cylindrical, slightly curved or straight in shape. There are a lot of microconidia that appears to be abundant. The macroscopic and microscopic characteristics showed that sample 5 is *Aspergillus* based on the microscopic morphological features of the *Aspergillus* [10]. *Aspergillus* sp. is a filamentous, cosmopolitan and ubiquitous fungus found in nature. *Aspergillus sp.* can withstand low temperature and it is commonly isolated from soil and plant debris.

Culture 6

Culture 6 has a slightly slow growth rate. The colonies are initially white and turn to greenish yellow. Reverse is pale yellow (Figure 11). The texture is cottony and granular. The microscopic examination shows that culture 6 has a complex hyphal structures which is hyaline hyphae with clamps and septate. Conidiophores are hyaline and unbranched (Figure 12). The phialides are hyphae, flask-shaped and may be solitary or arranged in clusters. The color of the conidia is green and round or ellipsoidal in shape. The conidia are appears in pairs or clusters.



(a) (b)

Figure 11: Macroscopic view of culture 6 (a) Front view (b) Back view

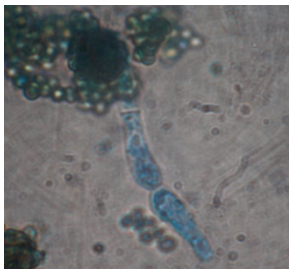
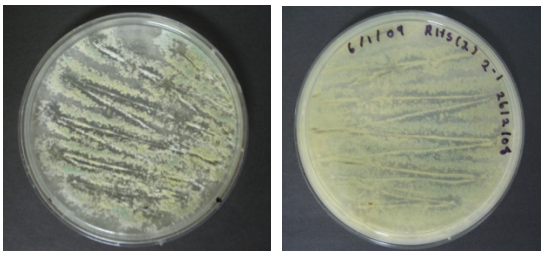


Figure 12: Microscopic view of culture 6 (400X magnification)

Culture 7

Culture 7 has a rapid growth rate. The texture is cottony and granular. The colonies are initially white and quickly turn greenish and becoming dark green with age. Reverse is pale yellow greenish (Figure 13). The hyphae are thin, hyaline and skeletal. The conidiophores are hyaline and unbranched. Phialide were presented and occurred in false heads (a collection of conidia at the tip of the phialide). Conidia appear green in color, one-celled, ovoid to cylindrical, accumulating in a single, terminal, large ball, or occasionally in a loose column (Figure 14). A penicillus bearing is a single, large, slimy ball of one-celled conidia which is typical for genus *Gliocladium*. The macroscopic and microscopic characteristics shows that sample 7 is a *Gliocladium* sp. based on the microscopic morphological features of the *Gliocladium* sp. [10]. Culture 7 was isolated from strawberry plant soil and *Gliocladium* sp. is a filamentous fungus which is widely distributed in soil for decaying vegetation and fruits [13].



(a) (b)

Figure 13: Macroscopic view of culture 7 (a) Front view (b) Back view

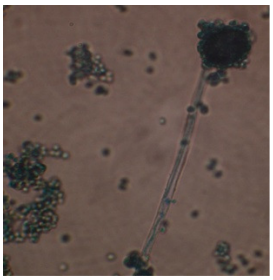


Figure14: Microscopic view of culture 7 (400X magnification)

CONCLUSION

In this research, seven cultures of fungi that were isolated from vegetables soil of Cameroon Highlands were successfully grown to obtain the pure culture for microscopic identification in which the genus of six cultures were successfully identified using macroscopic and microscopic approaches. Genus identified were *Aspergillus*, *Fusarium*, *Rhizopus* and *Gliocladium*. Further research should be done on molecular approaches for the species identification.

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