ORIGINAL ARTICLE

Isolation and presumptive identification of the major fungi associated with infestation of the Final Year Project Laboratory

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Abstract:

In 2017, severe fungal infestation occurred in an adjoining room in the Final Year Project Laboratory at FSK 1,5 UiTM, Puncak Alam. Abundant fungal growth was apparent on walls, ceilings, floor and even furniture. This project aimed to isolate and characterize the major contaminating fungi at the site. Fungal isolation was performed by swabbing the area of infestation using sterile cotton swab and directly spreading onto Sabouraud Dextrose agar, and then subcultured for pure colony growth. Macroscopic (phenotypic) characterization was performed by viewing colony characteristics under 10x and 40x magnifications using the light microscope. Microscopic characterization was performed by tease mount and slide culture methods. Based on macroscopic and microscopic observations of colony morphology characteristics, reproductive structures and growth rate on SDA plate, the two major types of fungi isolated were presumptively identified as *Aspergillus* spp. and *Rhizopus* spp.

Keywords: *Aspergillus* spp, fungal infestation, phenotypic criteria, *Rhizopus* spp

1. INTRODUCTION

Fungi comprises of a diverse group of heterotrophic cellulose decomposers that play an important role in the ecosystem [1] as they are saprophytes that digest dead organic matter which is most essential in carbon recycling [2]. They are also important facultative parasites in human causing opportunistic infections [3]. Environmental fungi, previously known contaminants, saprobes or plant pathogens are now recognized as opportunistic fungi which can cause harm especially to those with underlying medical disorders such as in immunocompromised patients [4]. Fungal contamination in a building is one factor that contributes to sick-building syndrome which adversely affects health [5-6]. Fungal infestations affect the structural integrity of a building which is facilitated by building environments that have low temperature, reduced lighting and high humidity that enhance fungal growth and sporulation to produce numerous enzymes and metabolites that are damaging to paint and building structure. Species of *Aspergillus*, *Penicillium*, *Cephalosporium*, *Cladosporium* and *Rhizopus* were commonly isolated from paints with fungal infestations [7]. Fungal contamination affects human olfactory systems with mycotoxins and allergens [8], especially in immunocompromised and highly sensitive individuals such as asthmatics [9-10]. Some environmental fungi are agents of opportunistic mycoses, which are human fungal diseases often caused by multiple organisms and comprises of subcutaneous, superficial and systemic infections [11]. Among all mycoses cases, the most critical worldwide problems are fungal infections of the nails and skin [12-13] and dermatophytes, yeasts and moulds were the frequent pathogens involved in superficial mycoses[12].

Fungal growth in laboratories, lecturers' rooms, toilets and buildings structures is persistent and was recurrent despite treatment with antifungal chemicals. In 2017, this problem was exceptionally severe in an adjoining room in the Final Year Project (FYP) Laboratory at the Centre of Medical Laboratory Technology, FSK 1,5 Faculty of Health Sciences UiTM, Puncak Alam. Abundant fungal growth infestations were apparent on walls, ceilings, floor and even furniture. This study, as part of the undergraduate final year project, aimed to isolate and characterize the major contaminating fungi at the site.

2. MATERIALS AND METHODS

Fungal isolation was performed by swabbing the area of infestation in the Final Year Project (FYP) laboratory at Medical Laboratory Technology (MLT) Centre, UiTM Puncak Alam using sterile cotton swab and directly spreading onto Sabouraud Dextrose agar (SDA). Students involved wore protective garments with gloves and face–masks while sampling. The second method was by air sampling, where the SDA plates were exposed in the lab overnight and collected the following day. Plates were incubated at room temperature (28°C) for 3 to 7 days [14] and inspected daily for growth. To obtain pure cultures, each prominent fungal type which grew on the initial isolation

plate was sub-cultured onto fresh SDA plate and incubated at room temperature (28°C) for 3-7 days in the dark [14]. All plates were sealed with Parafilm to protect from other contaminating fungi [15].

Presumptive identification of the most prominent fungal isolate(s) on SDA was done by two methods. Macroscopic (morphological) characterization was done by viewing colony characteristics at the front and reverse side of plate. Examinations included growth rate of the fungus, its general topography which was observed best on the reverse side of plates, colony texture observed in cross-section and pigmentation on the surface and reverse side of the plate [15]. Microscopic characterization was performed by tease mount and slide culture [15] and staining with Lactophenol Cotton Blue (LPCB) [16]. All morphological characteristics of fungus were then compared to the reference book [17] for presumptive identification.

The isolates were presumptively identified on the basis of macroscopic as well as mycelia and spore characteristics as compared to the reference book [17].

3. RESULTS AND DISCUSSION

Two major fungi were isolated on SDA which were presumptively identified. Both fungal isolates were prepared for identification by tease mount method or slide culture method and stained with LPCB stain for microscopic visualization. Colony morphologies of both fungi were observed under 10x and 40x magnifications using light microscope. Figures 1 and 2 show macroscopic morphologies of fungal isolate #1 (FG1) on SDA and its microstructures, respectively. FG1 (Figure 1) grew as a flat white colony with powdery brown conidia at the colony center. It was also white on the reverse side of the SDA plate and matured in 3 to 4 days. FG1 demonstrated characteristics of globose conidia that expressed singly or in groups, together with septate hyphae. The conidial head morphology was in uniseriate, where only single layer of phialides was formed. Most of the conidial head of FG1 were typically radiate. Labelled reproductive structures of FG1, as shown in Figure 2, demonstrate structures closely identified as *Aspergillus* spp.

Fungal Isolate #2 (FG2) (Figures 3 and 4) showed white dense cottony growth on surface of the SDA plate and was also white on the reverse side. As it matured in 6 to 7 days, the colony turned grey or yellowish brown from sporulation. FG2 demonstrated characteristic globose sporangiospores, where formation of sporangiospores was both in singles and in groups. Columella, an umbrella-like structure, was identified and there was presence of clear sporangiophore and rhizoids. Labelled structures of FG2 as shown in Figure 4 demonstrate structures closely identified as *Rhizopus* spp.

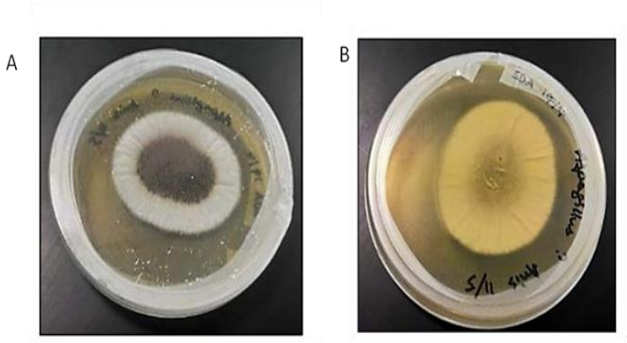

Figure 1: Macromorphology of fungal isolate #1(FG1) on SDA. A. Front view, B. Reverse view

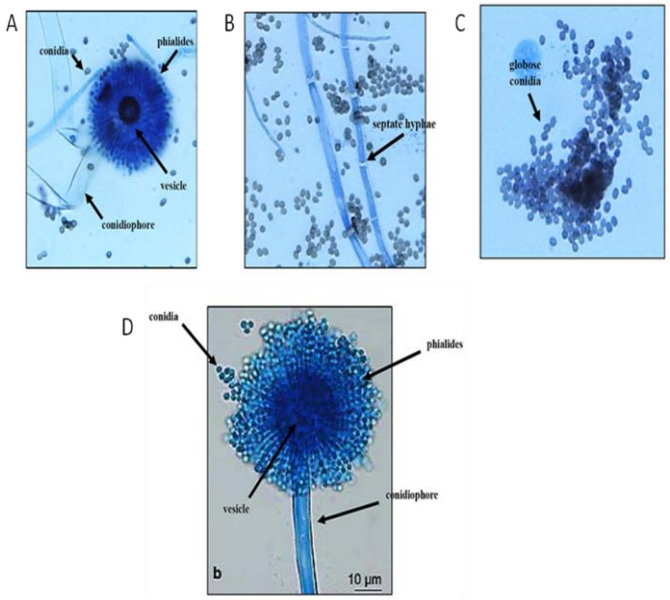

Figure 2: Microstructure of fungal isolate #1 (FG1).
A. Phialide, vesicle and conidiophore, B. Hyphae (septate), C. Conidia (globose), D. Reference structure *Aspergillus*

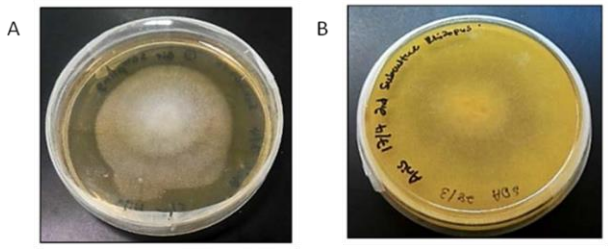

Figure 3: Macromorphology of fungal isolate #2 (FG2) on SDA. A. Front view, B. Reverse view

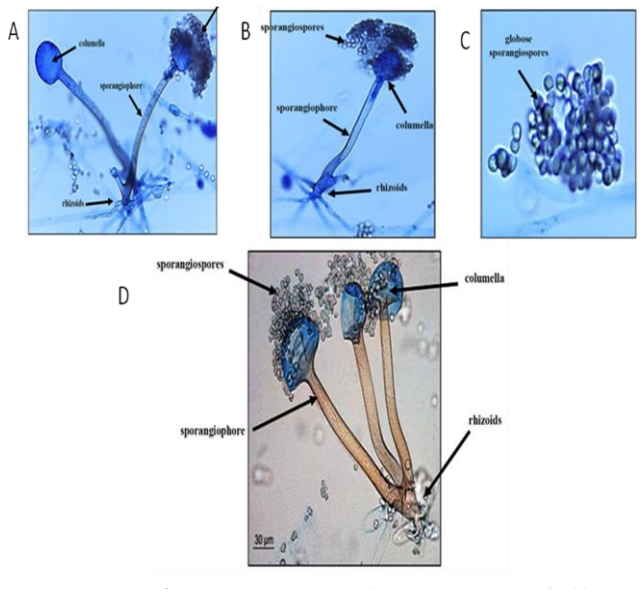

Figure 4: Microstructure of fungal isolate #2 (FG2).
A. Phialide, vesicle and conidiophore, B. Hyphae (septate), C. Conidia (globose), D. Reference structure of *Rhizopus*

Based on morphological observations and referencing to 'Descriptions of Medical Fungi' book [17] and growth rate on SDA, FG1 and FG2 were presumptively identified as *Aspergillus* spp. (Fungal isolate #1, FG1) and *Rhizopus* spp. (Fungal isolate #2, FG2), respectively.

3.1 Macroscopic

Morphological characterization of the isolated fungi was done by examining the fungal growth on SDA plates. The growth rate of the fungi, their general topography (reverse side of plates), the texture in cross section of the colonies and pigmentation were observed [15]. Presumptive identification was done by referring the growth rate, colony morphology and reproductive structures of both isolated fungi to the established morphological characteristics in reference text [17-18]. There are more than 250 recorded species for *Aspergillus* [19]. Basic and traditional identification of *Aspergillus* spp. has been mainly on phenotypic criteria based on macroscopic characterizations and microscopic observations [20]. Table 1 shows the summarized characteristics of fungal isolates, FG1 and FG2.

In addition to SDA, differential media such as Czapek Dox agar (CZ), Czapek Yeast agar (CYA), Malt extract agar (MEA) and Czapek yeast 20% sucrose agar can be used to identify *Aspergillus* species. Different species of *Aspergillus* will give different morphological characterizations. For example, on Czapek Dox agar, *Aspergillus flavus* shows a granular and flat colony which is yellow and will turn to bright dark yellow-green when matured. Meanwhile on the same agar, it was reported that *Aspergillus fumigatus* portrays a different phenotype, where the colonies

Table 1: Morphological characteristics of fungal isolate #1 and #2 on SDA

Morphological characteristics of fungi	#1	#2
Growth rate	Fast growing (mature in 3-4 days)	Fast growing (mature in 6-7 days)
Front side of SDA	• White colony • Flat colony with powdery brown conidia at center of colony	• White colony • Dense cottony growth colony that becomes grey or yellowish brown with sporulation when mature
Reverse side of SDA	White in color	White in color

demonstrated blue-green surface pigments with dense conidiophores. *Aspergillus niger* however possesses a compact white or yellow basal, with surface layered by black conidial heads. One of the isolated fungi in this study, FG1, was presumptively identified *Aspergillus* spp. as, it showed identical morphological characteristics to those of *Aspergillus* species on SDA as was reported previously [20]. It appeared as a white flat colony with powdery brown conidia at the center. On the reverse side of the plate, white colony coloration was observed. This fungus was a fast-growing fungus, where it matured in 3 to 4 days.

The other presumptively identified fungal isolate, FG2, showed identical morphological characteristics of *Rhizopus* species as was reported previously [17]. It portrayed a white colony on the surface and reverse side of the SDA plate with dense cottony growth that turned grey or yellowish brown with sporulation when matured. It is also a fast-growing fungus, which reached maturity in 6 to 7 days. The genus *Rhizopus* consists of three major clades, with *R. microsporus* as the basal species and the sister lineage to *R. stolonifer* and two closely related species *R. arrhizus* and *R. delemar* [21]. These three groups have different growth temperature characteristics, where 'oryzae' group grows best at 40°C, 'stolonifier' group at 30°C and the 'microsporus' group grew in range of 40°C to 45°C [17]. *Rhizopus* genus is identified by the existence of stolons and rhizoids pigmentation. Other characteristics of *Rhizopus* spp. recorded are the development of sporangiophores directly from rhizoids structure together with the presence of columellate and globose sporangia. Columella will form to umbrella-like structure after releasing the sporangiospores.

3.2 Microscopic

Identification of *Aspergillus* spp. based on macroscopic and microscopic characterizations has been previously proven to be relevant [20] as was performed in this study. Microscopic characterization of colony morphology was performed by observing colonies under low (10x) and high (40x) magnification using the light microscope.

Visualization of reproductive structures was aided by LPCB that consists of phenol, lactic acid, and cotton blue which serve to kill live organisms, preserve fungal structures and stain the chitin in fungal cell walls, respectively [16]. Most *Aspergillus* species possess similar characteristics microscopically with general structures that include having globose conidia, uniseriate or biseriate conidial head morphology, and also rough-wall conidiophore stipes connected directly to the vesicle. Only the shape, length of specific structures as well as the wall structure of stipe or conidia differentiates between species of *Aspergillus*.

However, limited studies have been published for *Rhizopus* spp. using macroscopic and microscopic characterizations. Most previous studies demonstrated identification of *Rhizopus* spp. using molecular analysis [21]. General structures of *Rhizopus* spp. possess simple pale-brown rhizoids, brownish sporangiophores together with greyish-black spherical sporangia with subglobose to globose columellae [17]. No specific differentiation was reported, as the differentiation of *Rhizopus* species were done by molecular analysis.

In this study, two types of fungi were presumptively identified based on microscopic characterization methods, which were *Aspergillus* spp. and *Rhizopus* spp. Presumptive identification was done by referring to the established fungal structures of both isolated fungi in the reference book, as shown in Figures 2D and 4D that demonstrated structures of *Aspergillus flavus* and *Rhizopus microsporus* structures, respectively [17]. However, both isolated fungi were only presumptively identified to the genus level. There were limitations in identifying specific species of both fungi as no biochemical sets were available for the purpose. Molecular analysis was not performed due to time and financial constraints for this undergraduate project. In the clinical laboratories, mold identification by comparative sequence analysis reduces subjectivity compared to identification using phenotypic criteria [22].

The fungal infestation at the aforementioned laboratory has since been contained with effective installation of proper ventilation and lighting units. Noteworthy, it is necessary to maintain continuous operation of the air-conditioning units in the laboratory, irrespective of use. Discontinuation of the air-conditioning units seems to have exacerbated the problem due to high humidity and conducive temperature for fungal growth in the area.

4. CONCLUSION

Based on macroscopic and microscopic observations of colony morphology characteristics, reproductive structures and growth rate on SDA plate, the two types of fungi that were isolated from infestation in the Final Year Laboratory were presumptively identified as *Aspergillus* spp. and *Rhizopus* spp.

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