ORIGINAL ARTICLE

Identification of airborne fungi isolated from Faculty of Dentistry in Universiti Teknologi MARA Sungai Buloh Campus

Nurina Hannani Hishammuddin[a], Hartini Yusof[a], Nurul 'Izzah Mohd Sarmin*[b,c]

[a]Centre of Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA(UiTM), UiTM Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia; [b]Centre of Preclinical Science Studies, Faculty of Dentistry, Universiti Teknologi MARA (UiTM), UiTM Kampus Sungai Buloh, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia; [c]Atta-ur-Rahman Institute for Natural Product Discovery, UiTM Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia

***Corresponding Author**

Nurul 'Izzah Sarmin, PhD
Email: izzahsarmin@uitm.edu.my

Abstract:

Indoor airborne fungi are known to be the contributor to irritative disorders, primarily non-infective diseases such as allergy and asthma. Spores and hyphal fragments of fungi contained in bioaerosols can be breathed in easily and evoke bronchial irritation and allergy and in immunocompromised individuals, fungi can cause opportunistic infections. Therefore, the present study was undertaken to identify the airborne fungi that have been isolated from the building of Faculty of Dentistry in UiTM Sungai Buloh campus by using macromorphology, micromorphology and also molecular study. Fungi that were kept in 20% glycerol stock were subcultured on Sabouraud dextrose agar (SDA) and were observed for their macromorphological and micromorphological characteristics. The macromorphology of the fungal isolates were observed based on colour, texture and form while the micromorphology were observed for morphological structures such as phialides and metulae. Subsequently, DNA was then extracted and the internal transcribed spacer regions (ITS1-5.8S-ITS2) was amplified using forward primer ITS1 and reverse primer ITS4. One of the amplicons were sent for sequencing and was identified as *Penicillium citrinum* with 99% sequence similarity. In conclusion, identification of microorganisms isolated from dental building is necessary to minimise cross infection to the patients and healthcare providers.

Keywords: Airborne fungi, identification, ITS, molecular, PCR

1. INTRODUCTION

Fungi are living microorganisms that are present in various places and natural surroundings including freshwater and sea, waste, decaying remains of plants and animals, as well as in living plants and animals [1]. Despite their abundance in the environment, food products, gastrointestinal tract and skin, very few yeasts and fungi trigger disease in healthy people [2]. Fungi which includes yeasts, moulds and mildews can cause opportunistic infections to immunocompromised individuals [3]. Immunocompromised individuals will have an increase risk to develop fungal infection as the fungi present in the environment may be opportunistic and pathogenic to them.

In dental settings, many dental procedures such as grinding, drilling, air-abrasion polishing and ultrasonic scaling can contribute to generation of aerosol and bioaerosol particles, causing increased concentration of relatively large particles in the air [4]. During the procedures where ultrasonic scaling or high-speed drill are being used, aerosols and splatters concentration appears to be the highest and the bioaerosols can exist in the air up to few hours if there is insufficient air ventilation [5]. Surfaces and equipment such as chair, spotlight and dental equipment might also be contaminated by the aerosols produced during dental procedures. In previous study, there were 14 species of fungi isolated from dental chairs, which have been identified belonging to the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*, *Fusarium*, *Curvularia*, *Drechslera* and *Paecillomyces* [6]. Besides dental procedures, the proliferation and spreading of fungi in dental settings is also contributed by internal air conditioning [7].

Staffs and students of the Faculty of Dentistry, UiTM Sungai Buloh and the patients who visit the dental clinics have the risk to develop fungal infection as fungi exist in its environment. Due to the threat from opportunistic and pathogenic fungi, the identification of fungi isolated from the air environment must be done periodically.

2. MATERIALS AND METHODS

2.1 Sample collection

Fungal samples were collected from the air in the dental buildings by previous student and were kept in 20% glycerol stock stored at -20°C. The samples were collected from four different locations at the Faculty of Dentistry, which were at the dental clinic, laboratories, lecturers' room and preparation room.

2.2 Culturing fungi on Sabouraud Dextrose agar (SDA)

A total of four fungal isolates that were kept in 20% glycerol stock were subcultured on SDA and were incubated at 30°C for 3 to 7 days. The four fungal isolates were the ones with code name of Lecturer Room PM (2nd) (1), Pro Lab 1AM (2nd) #2, Pro Lab 1PM (2nd) (2) and Spinel ER PM SP.2 #2 and they were labelled as Isolate 1, Isolate 2, Isolate 3 and Isolate 4, respectively.

2.3 Macromorphological observation of fungal isolates

Observation of colony morphology of the fungal isolates were done based on colour, texture and topography of the colonies and the characteristics were recorded.

2.4 Micromorphological identification of fungal isolates

Microscopic identification was done by performing lactophenol cotton blue (LPCB) staining and observation under the microscope. One drop of LPCB stain was placed in the center of a clean slide. A fragment of the fungus colony was removed by using sterile toothpicks. The fragment was placed in the drop of stain and was teased gently. Coverslip was applied before observing the slide under microscope.

2.5 Molecular characterisation of fungal isolates

Initially, DNA from the fungal isolates were extracted using thermolysis method. Fungal DNA had also been extracted using NucleoSpin Microbial DNA kit protocol. The purity and quality of the extracted fungal DNA were assessed by means of 0.8% agarose gel electrophoresis. The bands obtained were visualized using a gel documentation system, ImageQuant LAS 500.

Internal transcribed spacer (ITS) region of fungal rRNA genes was amplified using the following universal primer set: forward ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and reverse ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The amplification program had two protocols with two different annealing temperature. Protocol I: initial denaturation at 95°C for 5 minutes, 35 cycles of 95°C for 1 minute, annealing at 50.8°C for 1 minute, and extension at 72°C for 1 minute, and then final extension for 10 minutes at 72°C. Protocol II: initial denaturation at 95°C for 5 minutes, 40 cycles of 95°C for 1 minute, annealing at 50.7°C for 1 minute, and extension at 72°C for 1 minute, and then final extension for 10 minutes at 72°C. The PCR products were detected by electrophoresis on a 1% agarose gel.

The amplified PCR products were sent for sequencing. Each sample was sequenced in the sense and antisense directions using ITS1 and ITS4 primers. The nucleotide sequences were then analysed using BLAST from the National Center of Biotechnology Information (NCBI) website [8].

3. RESULTS AND DISCUSSION

3.1 Macromorphological and micromorphological characteristics of fungal isolates

In identifying fungi, the morphological features of the fungal colonies are observed [4, 9, 10, 11]. Morphological features include macroscopic appearance of fungal colonies on growth medium and microscopic view of the fungi when observed under the microscope.

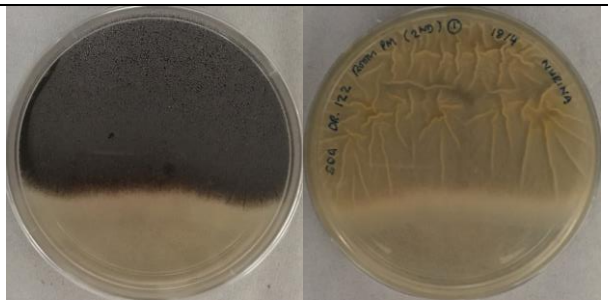
Macromorphology

Front side Reverse side
Description
• Black, powdery colony with white mycelium • Reverse side is white

Figure 1: Macromorphological characteristics of isolate 1

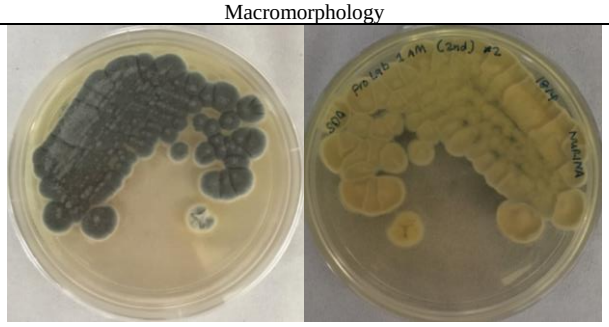
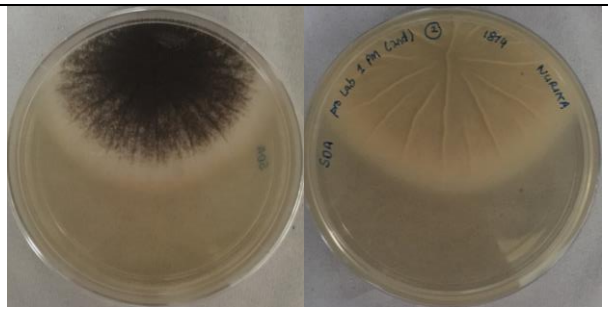
Macromorphology

Front side Reverse side
Description
• Olive green colony with velvety texture and rugose appearance • Reverse side is yellow

Figure 2: Macromorphological characteristics of isolate 2

For isolate 1, based on its macromorphological characteristics in Figure 1, the colony was black and powdery with white mycelium and the reverse side of the SDA plate was white. This fungus was presumptively identified as *Aspergillus niger* as it bare black conidia growing from its white mycelium [12]. As for its microscopic characteristics (Table 5), stipe and vesicle can be observed but not metulae and phialides as they were obscured by conidia.

Macromorphology



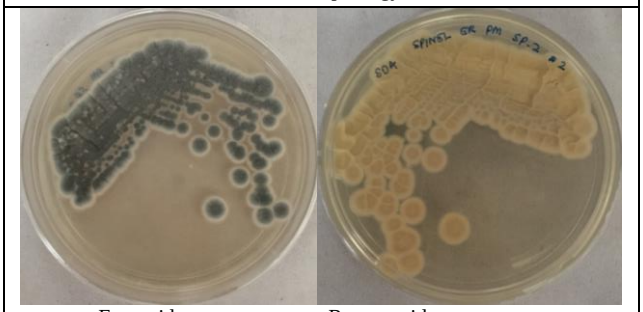
Front side Reverse side

Description

- White colony with black pigmentation, powdery texture and rugose appearance
- Reverse side is white

Figure 3: Macromorphological characteristics of isolate 3

Macromorphology



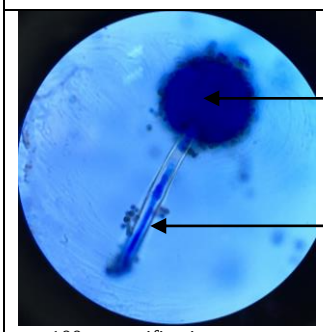
Front side Reverse side

Description

- Dark green colony with white border, waxy texture and rugose appearance
- Reverse side is yellow

Figure 4: Macromorphological characteristics of isolate 4

Micromorphology

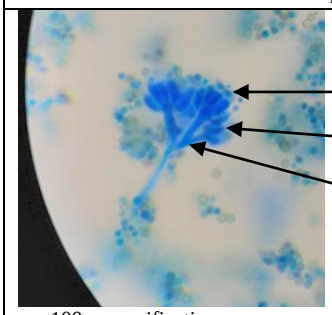


100x magnification

- Heavy conidiation obscures metulae and phialides.

Figure 5: Micromorphological characteristics of isolate 1

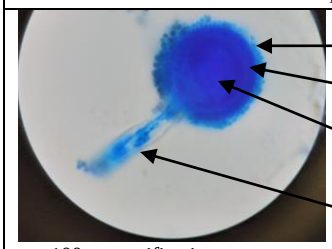
Micromorphology



100x magnification

Figure 6: Micromorphological characteristics of isolate 2

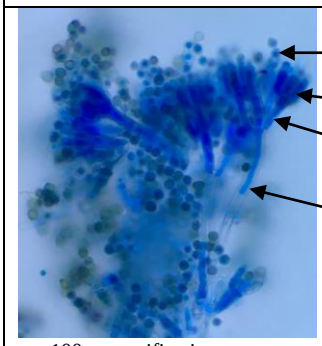
Micromorphology



100x magnification

Figure 7: Micromorphological characteristics of isolate 3

Micromorphology



100x magnification

Figure 8: Micromorphological characteristics of isolate 4

For isolate 2, as described in Figure 2, the colour of the colony was olive green with velvety texture, rugose appearance and yellow reverse side. Its microscopic view (Table 6) showed phialides, metulae and conidia. A brush-like appearance (penicillus) that can be observed under the microscope was the result of production of phialides in groups from branched metulae (a penicillus) [12]. This fungal isolate was presumptively identified as *Penicillium* spp. and molecular identification by ITS sequencing has confirmed it.

For isolate 3, as described in Figure 3, the colony was white but almost covered in black, has powdery texture and rugose appearance. The reverse side of the SDA plate was white. The microscopic view (Table 7) showed the structures like stipe, vesicle, phialides and conidia. This fungal isolate was

also presumptively identified as *Aspergillus niger* as it produced black conidia from its white mycelium [12].

For isolate 4, based on its macromorphological characteristics in Figure 4 and microscopic characteristics in Figure 8, it was presumptively identified as *Penicillium* spp. as the colony was in shade of green with white border, waxy texture and rugose appearance. The microscopic view of this fungus also vividly showed brush-like structure (penicillus), which justifies that this fungus was from the genus *Penicillium*.

Based on morphological features of those four fungal isolates, presumptively, it can be said that Isolate 1 and Isolate 3 were from the same fungal genus, *Aspergillus* and Isolate 2 and Isolate 4 were from the same fungal genus, *Penicillium*, as they have similarities in the colony appearances and microscopic structures. However, this cannot be confirmed without molecular identification as it was challenging to identify the fungi solely based on their morphology since morphological characters that can be used for identification are limited [13].

3.2 Molecular identification of fungal isolates

PCR was carried out using Protocol I and Protocol II. There were no bands shown on 1.0% agarose gel that can be observed when using Protocol I (not shown). This may be due to insufficient number of cycles for the amplification to occur due to low concentration of DNA template. There was only one band that can be observed for PCR product using Protocol II which was the product of isolate 2 (not shown). The band was faint, which was possibly caused by low concentration of DNA in the PCR product. Therefore, quantity of DNA loaded into the gel was insufficient. Despite that, the PCR product was sent for DNA sequencing to identify the species of fungal isolate 2. It was found that the sequencing results received for both forward and reverse primers ITS1 and ITS4 were low in quality due to high in content of base G and C, hence it was sent for sequencing again with specific requirement for high GC. The nucleotide sequence received was then compared to nucleotide sequences in the NCBI's GenBank database using BLAST search. The nucleotide sequence of isolate 2 showed 99% similarity with *Penicillium citrinum*. Any sequencing result should have ≥97% to 100% sequence similarity and ≥80% query coverage to be considered as the same species [13].

Penicillium citrinum has rarely been reported to cause infection in humans. However, there were cases in which *P. citrinum* caused cutaneous penicilliosis in a child with acute myeloid leukaemia [14] and fatal pulmonary infection in an immunocompromised patient [15]. This identifies that *P. citrinum* is an opportunistic airborne fungus which can cause infection in individuals with weakened immune system.

The result of the present study was supported by a previous study in which seven out of 104 fungal isolates that have been isolated from the air of a dental healthcare environment were identified as *Penicillium* spp. [16]. *Penicillium* spp. was also predominantly found in the air of other environments besides dental settings [17, 18]. Inhalation of *Penicillium* spp. spores can cause hypersensitivity pneumonitis, allergic rhinitis and allergic asthma in sensitive person [19]. Therefore, any occupants of the environments containing these fungal spores are prone to develop allergies and respiratory diseases.

4. CONCLUSION

Airborne fungi are present in the environment of dental healthcare settings and combination of morphological study and molecular study is useful for identification of the airborne fungi. One of the airborne fungi that had been isolated from the environment of the Faculty of Dentistry, UiTM Sungai Buloh campus was identified as *Penicillium citrinum*, which was identified as an opportunistic fungi to the immunocompromised individuals. From this discovery, the healthcare providers, students and patients of this institution are likely to be infected with the airborne fungi when their immune system are weak. Therefore, it is important to take preventive measures and do periodic indoor air monitoring to control the spreading of opportunistic fungi to the healthcare providers, students and patients and to prevent fungal contamination.

ACKNOWLEDGEMENT

The authors are grateful for the fund provided by Lestari grant [600-IRMI/DANA 5/3/LESTARI (0069/2016)].

REFERENCES

[1] Dix, N. J., and Webster, J., "Introduction" in *Fungal Ecology*, First edit., Dordrecht: Springer Netherlands, 1–11, 1995.

[2] Rizzetto, L., and Cavalieri, D., "Friend or foe: Using systems biology to elucidate interactions between fungi and their hosts." *Trends Microbiol.*, 19(10): 509–515, 2011.

[3] Collins, A. S., Cleveland, J. L., and Harte, J. A., "Guidelines for Infection Control in Dental Health-Care Settings," vol. 52, 2003.

[4] Polednik, B., "Aerosol and bioaerosol particles in a dental office." *Environ. Res.*, 134: 405–409, 2014.

[5] Gowtham, S., and Padma, E. D., "Bioaerosol contamination in dental clinic: a potential health hazard?." *Ann. Essences Dent.*, 6(1): 33, 2015.

[6] de Almondes, A. I. V., *et al*., "Fungal contamination and disinfection of dental chairs, Teresina, Piaui, Brazil." *Acta Odontol. Latinoam*, 29(3): 225–229, 2016.

[7] de Sousa, K. S., and Fortuna, J. L., "Microorganisms in Air-conditioned Dental Offices in a Southern Town of Bahia." *Rev. Baiana Saúde Pública*, 35(2): 250–263, 2011.

[8] Nasser, L. A., "Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia." *Saudi J. Biol. Sci.*, 22(5): 513–520, 2015.

[9] Duncan, S. M., *et al*., "Monitoring and identification of airborne fungi at historic locations on Ross Island, Antarctica." *Polar Sci.*, 4(2): 275–283, 2010.

[10] Kim, K. Y., Kim, Y. S., and Kim, D., "Distribution Characteristics of Airborne Bacteria and Fungi in the

General Hospitals of Korea." *Ind. Health*, 48(2): 236–243, 2010.

[11] Kowalski, M., *et al.*, "Characteristics of airborne bacteria and fungi in some Polish wastewater treatment plants." *Int. J. Environ. Sci. Technol.*, 14(10): 2181–2192, 2017.

[12] Kidd, S., *et al*., *Descriptions of Medical Fungi*, 3rd ed., Adelaide: CutCut Digital, 2016.

[13] Raja, H. A., *et al*.,"Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community." *J. Nat. Prod.*, 80(3): 756–770, 2017.

[14] Thoon, K., C., *et al.*, "A case of cutaneous penicilliosis in a child with acute myeloid leukaemia." *JMM Case Reports*, 2(5): 1–4, 2015.

[15] Chowdhary, A., *et al.*, "Voriconazole-Resistant Penicillium oxalicum: An Emerging Pathogen in Immunocompromised Hosts." *Open Forum Infect. Dis.*, 1(2): 1–7, 2014.

[16] Vieira, C., D., *et al*., "Isolation of clinically relevant fungal species from solid waste and environment of dental health services." *Lett. Appl. Microbiol.*, 51(4): 370–376, 2010.

[17] Baurès, E., *et al.*, "Indoor air quality in two French hospitals: Measurement of chemical and microbiological contaminants." *Sci. Total Environ.*, 642: 168–179, 2018.

[18] Cabo Verde, S., *et al*., "Microbiological assessment of indoor air quality at different hospital sites." *Res. Microbiol.*, 166: 557–563, 2015.

[19] Simon-Nobbe, B., et al., "The Spectrum of Fungal Allergy," *Int. Arch. Allergy Immunol.*, 145(1): 58–86, 2008.