Songklanakarin J. Sci. Technol.
42 (6), 1294-1303, Nov. - Dec. 2020

http://www.sjst.psu.ac.th

Short Communication

Microstructures of *Aspergillus* spp. and their role in contaminating clinical solid wastes

Efaq Noman[1, 2*], Adel Al-Gheethi[3], Nik Norulaini Nik Ab. Rahman[4], Nagao Hideyuki[5], Balkis A Talip[2], Radin Mohamed[3], and Mohd. Omar Ab. Kadir[6]

[1] *Department of Applied Microbiology, Faculty of Applied Sciences, Taiz University, Taiz, Yemen*

[2] *Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Pagoh, Johor, 84000 Malaysia*

[3] *Division of Water and Environmental Engineering, Faculty of Civil Engineering and Built Environment, Universiti Tun Hussein Onn Malaysia, Batu Pahat, Johor, 86400 Malaysia*

[4] *School of Distance Education, Universiti Sains Malaysia, Penang, 11800 Malaysia*

[5] *School of Biological Science, Universiti Sains Malaysia, Penang, 11800 Malaysia*

[6] *School of Industrial Technology, Universiti Sains Malaysia, Penang, 11800 Malaysia*

Received: 6 June 2019; Revised: 6 August 2019; Accepted: 9 September 2019

Abstract

The aim of the current study was to describe the ultrastructure of *Aspergillus* spp. spores and conidiophores using Scanning Electron Microscope (SEM) and their role in contaminating of clinical solid wastes. *Aspergillus* spp. were described on, Czapek Yeast Extract Agar (CYA), Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA), Czapek-Dox Agar (CZ) and Potato Dextrose Agar (PDA), while the ultrastructure of spores and conidiophores were observed using light Microscope and SEM. Fungal isolates were identified as *A. caesiellus, A. terreus var. terreus; A. flavus, A. parasiticus, A. sydowii* and *A. Fumigatus* with two new strains identified as *Aspergillus* sp. strain no. 145, *Aspergillus* sp. strain no. 311. SEM analysis for *Aspergillus* spp. conidiophores and spores were more useful in the taxonomy of fungi. Microstructures of the spores play important role in the contamination of clinical wastes, where the spiny fungal spores surface have high adherence for the solid wastes.

Keywords: *Aspergillus* spp**.** SEM, microstructure, new strains, clinical wastes

1. Introduction

Clinical wastes term is used to identify the wastes generated from the medical establishments such as hospitals and clinical centres (World Health Organization [WHO], 2005). These wastes have high load of infectious agents such as bacteria, fungi and viruses (Efaq *et al.*, 2015). However, the simple growth requirements in terms of water activity (a_w) compared to bacteria increase the health risk. In addition, the fungal spores are easily spread to the environment and then transfer to animal and human (Nielsen *et al.*, 1999; WHO, 2009). The most common fungi which have been detected in the clinical solid wastes are *Fusarium* sp., *Paecilomyces* sp.,

*Corresponding author
Email address: norulain@usm.my; eanm1984@gmail.com

Scytalidium sp., *Cladosporium* spp., *Acremonium* spp., *Scopulariopsis* spp., *Basipetospora* spp., *Mucor* sp., *Curvularia* spp., *Aspergillus* sp., *Alternaria* sp., *Aureobasidium* sp., and *Penicillium* spp. (Neely & Orloff, 2001; Şahil & Otag, 2013; Vieira *et al.*, 2010;). Noman, Al-Gheethi, Rahman, Nagao, and Kadir (2016) have listed the isolated fungi from the solid clinical wastes in Malaysia. However, the same study has not reported the morphological characteristics of those listed fungi.

The identification of fungi by phenotypic method is depend on the culture and microscopic morphologies simultaneously (Noman *et al.*, 2018; Promputtha, Jeewon, Lum yong, McKenzie, & Hyde, 2005). Microscope characteristics include the morphologies of hyphal structures and color as well as spore size, shape and surface ornamentation which are used as key for the fungal identification. The phenotypic method is more useful to identify *Penicillium* spp., *Aspergillus* spp. *Curvularia* spp. *Rhizopus* spp. and *Trichoderma* spp. to a species level (Kumara & Rawal, 2008). However, the phenotypic characteristics of fungi are varied according to the culture media and incubation conditions (Guarro, Gené, & Stchigel, 1999). Hence, the authors suggested using different culture media for the identification of the fungal isolates by phenotypic method. Moreover, Scanning Electronic Microscopy (SEM) is an efficient technique for recognizing the ultrastructure of conidiophore and spores and then to get the accurate name of the fungal species (Cole & Samson, 1979; Guarro, Gené, & Stchigel, 1999). In some fungal species, SEM imaging might be equivalent to the molecular technique, For instance, two varieties of *C. lunata* has been recognized by using SEM, *C. lunata var*. *aeria* appeared to have a smooth conidia and stromata in culture, while *C. lunata var. lunata* exhibited a smooth to roughly conidia but without stromata in culture (Ellis, 1971; Sivanesan, 1987). The molecular analysis of 16S rRNA sequencing for both varieties revealed that these varieties have different sequences and then they were deposit in Genebank as *C. lunata* and *C. area* (Nakada, Tanaka, Tsune waki, & Tsuda, 1994; da Cunha *et al.*, 2013). The classification and identification of *Aspergillus* spp. has been investigated based on phenotypic characters, molecular and chemotaxonomic characterization. In the phenotypic method, the phialides (spore producing cells) arrangements on the vesicles surface are the key for the identification process. The phialides might cover the vesicle surface partially (columnar vesicles) or the entire vesicle surface (radiate head) and phialides are attached to the vesicle directly (uniseriate) or via metula (biseriate) (Robert, János, & Christian, 2011; Silva *et al.*, 2011; Jensen *et al.*, 2013). The present study focused on the description of *Aspergillus* spp. isolated from the clinical wastes. The culture and microscopic characteristics as well as microstructure of the conidiophores and spores were recognized by using different culture media and SEM.

2. Methodology

2.1 Recovering *Aspergillus* spp. from clinical solid waste samples

The clinical waste samples were collected from Wellness Centre at Universiti Sains Malaysia (USM), Penang, Malaysia in the period between May and December 2014 as described in previous work (Efaq, Al-Gheethi, Rahman, & Nagao, 2019). These samples include tissue papers, gloves, cotton, gauze, pasture pipette, needles kits, strips, yellow tips, wood sticks and microscopic slides**.** One piece of each sample was placed on V8 agar medium**.** Plates were incubated for 7 days at 28 ºC. The grown *Aspergillus* spp**.** was purified using a single spore isolation method as described by Noman, Al-Gheethi, Rahman, Nagao, and Kadir (2016).

2.2 Description and Identification of *Aspergillus* isolates

Purified *Aspergillus* spp. was sub-cultured on V8 agar, CZ, MEA, CYA and PDA. The colony size **(**Diameter, mm**)**, texture of the grown colony for each fungal isolate were recorded after 7 days at 28 ºC. Meanwhile, the hyphal structure and color as well as spore shape and size (25 spores were selected for each fungal isolate) were recorded using light microscope. Moreover the ultrastructure of conidiophores and spore shapes as well as orientation was determined using SEM as described in previous work (Noman *et al.*, 2018). The recorded culture and microscopic characteristics were compared to that reported by Barnett and Hunter (1998), Wata nabe (2002); Campbell, Johnson, and Warnock (2013); Robert, János, and Christian **(**2011**)**; Samson *et al.* (2010); Samson, Houbraken, Thrane, Frisvad, and Andersen (2007), Silva *et al.* (2011); National Mycology Reference Centre (NMRC, 2015) for the identification process. The identification process was illustrated in Figure 1.

3. Results and Discussion

The culture characteristics of grown colonies of *Aspergillus* spp. recovered from the clinical solid waste samples are illustrated in Table 1. It was noted that the fungal isolates exhibited different characteristics in the different culture media. *A. fumigatus* occurred with light green to grey on CZ, while grew as velvety to sulcate on CYA with blue color. In comparison, On CZ *Aspergillus* sp. strain no. 311 have dark green colonies, while appeared as a sulcate with wrinkled growth with dark and light green structure in the center on CYA (Figure 2a). The results of light microscopic examination revealed that *A. fumigatus* spore size was between 1.9 and 2.9 with average 2.4 µm, while was between 2.2 and 3.3 µm (mean 2.8 µm) in *Aspergillus* sp. strain no. 311 (Table 2). The ultrastructure analysis using SEM revealed that the fungal isolates have short and conidiophores, columnar head, round vesicle with uniseriate phialides. However, the *A. fumigatus* spore textures were globular in shape, spiny texture echinulate ornamentation and have a wrinkled surface (Figure 2b). In contrast, *Aspergillus* sp. strain no. 311 has globular spores with smooth surface, distinctly wrinkled in texture as well as warty ornamentation (Figure 2c).

On the investigated culture media similar colony characteristics (small colonies) were observed for *A. sydowii* and *Aspergillus* sp. strain no. 145 (Figure 3a, Table 1). They are grown with slight differences in their culture characteristics, green colonies with white edge were detected on CZ. *A. sydowii* has grown with white edge colonies on V8A. Both fungal isolates have colonies with white colour on CYA medium. *Aspergillus* sp. strain no. 145 has a smooth and narrow conidiophore; the vesicle was irregular, columnar head with biseriate phialides and branched metula**.** The spores were

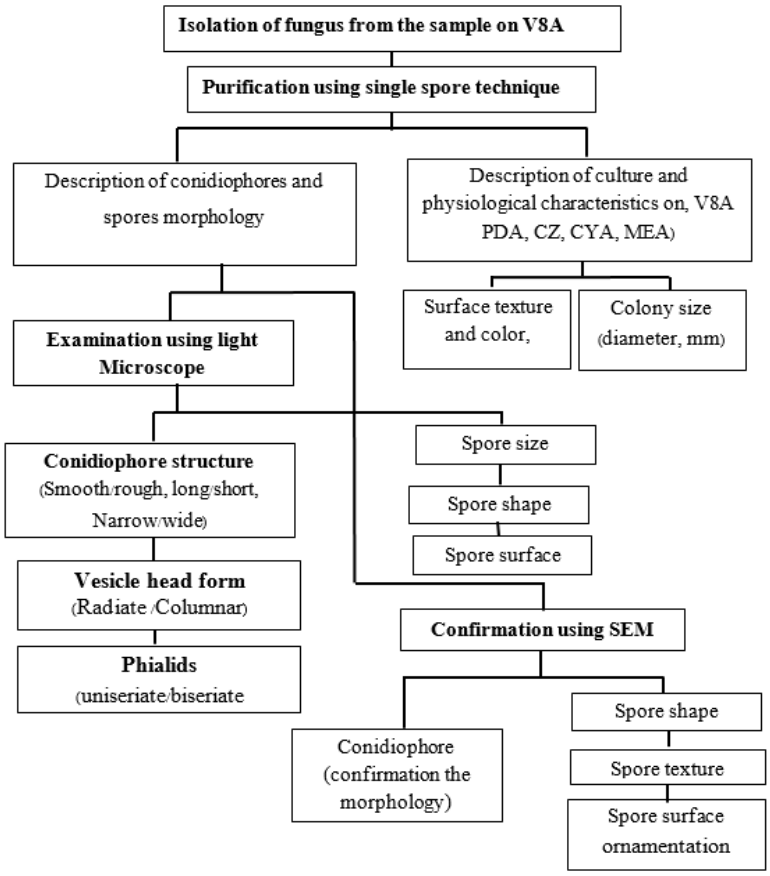

Figure 1. Flowchart of the identification process of *Aspergillus* spp.

Table 1. Culture characteristics of *Aspergillus* spp. isolated from clinical solid wastes after 7 days at 28 °C.

Fungus	Media type	Colony diameter (mm)	Colony character				
			Source of isolation	Texture	Surface color	Zonation (Margin)	Sporulation
A. fumigatus	V8A	66±4.3	Blood waste, gloves, HB cuvette and microscope slides wastes of haematology section - Cotton, gauze, and sharps wastes of emergency section, - Gauze, gloves and kits wastes of urine section - Gloves wastes of labeling section - Air of the storage room	velvety/sulcate	blue to olive	white	high
	CZ	28.6±3.9		crisp	light green/grey	white	high
	CYA	47±1.5		velvety/sulcate	blue	white	high
	MEA	48±5.9		velvety/sulcate	blue	white	high
	PDA	48±4.2		annular thin	dark green to grey	white	high
	SDA	55±2.5		powdery	dark green	grey	high
Aspergillus sp. strain no.311	V8A	66±4.3	Gloves wastes of labeling section and air of the storage room	velvety	blue to olive	green	high
	CZ	25.5±5.5		floccose in center and wrinkled in edge	dark green	white	high
	CYA	28±3.7		sulcate wrinkled	dark green with light green in center	white	mo-derate
	MEA	28±6.5		floccose/wrinkled	dark green	white	high
	PDA	36±3.2		thin floccose	green with light blue center	white	high
	SDA	55±2.5		powdery	dark green	grey	high

Table 1. Continued.

Fungus	Media type	Colony diameter (mm)	Colony character				
			Source of isolation	Texture	Surface color	Zonation (Margin)	Sporulation
Aspergillus sp. strain no. 145	V8A	11±2.5	- Gloves and yellow tips wastes of haematology section	amaranthine	grey center	white	moderate
	CZ	5±1.3	- Gauze and sharps wastes of emergency section - Urine strips wastes,	amaranthine	green/grey center	white	low
	CYA	18±3.4		velvety/sulcate	white	white	moderate
	MEA	12±2.5	- Gloves wastes of labeling section	velvety/sulcate	green/white center/ clear exudate	white	moderate
	PDA	10±1.3		velvety	grey/light yellow center	white	moderate
	SDA	12±2.5		velvety	green	white	moderate
A.sydowii	V8A	11.3±2.5	Gloves wastes of haematology Section	amaranthine	grey	white	moderate
	CZ	4.1±1.3		amaranthine	dark green	white	moderate
	CYA	5±1.3		velvety/sulcate	white	colourless	low
	MEA	10±3.2		velvety	dark green	white	moderate
	PDA	8±2.5		velvety/annular	dark grey	white	moderate
	SDA	13±2.5		velvety	green	white	moderate
A. parasiticus	V8A	76±3.4	- Glucose lancet wastes of haematology section - Air of the storage room	floccose	dark green	yellowish	high
	CZ	30±5.6		thick floccose	light green color	white	moderate
	CYA	35±1.5		thick floccose wrinkled	white to creamy	white	low
	MEA	47±7.3		thick floccose center/sulcate edge	white with dark green center	white	high
	PDA	59±9		thin floccose/ crispy	light greenish/ yellowish	white to yellowish	high
	SDA	45±2.3		thin floccose	green	greenish	high
A. flavus	V8A	78±3.7	Air of the storage room	amaranthine/crisp	dark green	white	high
	CZ	27±2.1		thin floccose	green to olive	white	high
	CYA	71±2		floccose center/amaranthine with sulcate edge	green with yellowish edge	white	high
	MEA	40±3		floccose/ cotton like growth	white with green center	white	moderate
	PDA	75±4.2		floccose/ annular	green yellowish	greenish	high
	SDA	80±0.0		thin floccose	green	white	high
A. terreus var. terreus	V8A	45±6.8	HB cuvette, microscope slides, wastewater wastes of haematology section	powdery and radially in center	brown	white	high
	CZ	30±2.3	- Sharps wastes of emergency section - Kits and HGC kits wastes of urine section - Gloves wastes of labeling section	wrinkled	brown center	white to creamy	low
	CYA	32.3±7.3		wrinkled annular	brown colony with yellow center	white	moderate
	MEA	30±4.5	Air of the storage room	floccose annular	brown and yellow	white	moderate
	PDA	26±5.0		floccose	Light yellow to creamy	white	low
	SDA	55.5±1.5		powdery	brown	white	high
A. caesiellus	V8A	38±4.9	- Blood waste, HB cuvette and wood stick wastes of haematology section	floccose/ crisp	green center	white	moderate
	CZ	32±3.8	- HGC Kits wastes of urine section - Gloves wastes of labeling section	floccose	Green/yellow center	white	high

Table 1. Continued.

Fungus	Media type	Colony diameter (mm)	Colony character				
			Source of isolation	Texture	Surface color	Zonation (Margin)	Sporulation
	CYA	31±4.25		floccose	white	white	mo-derate
	MEA	64±1.3		cotton like growth	white	white	low
	PDA	38±4.7		floccose/crisp	dark green center	white	mo-derate
	SDA	67.5±2.5		floccose/annular	dark green center	white	high

V8 juice agar (V8A); Czapek-Dox *Agar* (CZ); Czapek Yeast Extract A*gar* (CYA); Malt Extract Agar (MEA); Potato Dextrose Agar (PDA); *Sabouraud dextrose* agar (SDA).

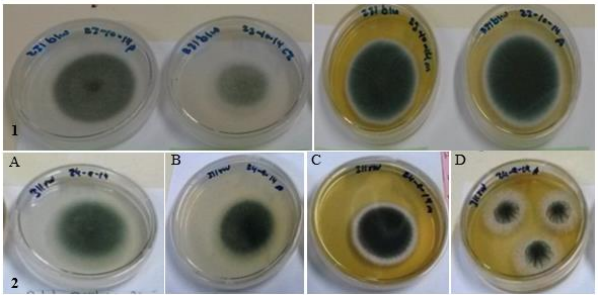

Figure 2a. Culture characteristics of *A. fumigatus* (No.1) and *Aspergillus* sp. strain no. 311 (No. 2). Both fungi were isolated from clinical solid wastes collected from USM health center on different culture media. The isolates were incubated at 28˚C for 7 days; A) Potato Dextrose Agar (PDA); B) Czapek-Dox *Agar* (CZ); C) Malt Extract Agar (MEA); D) Czapek Yeast Extract A*gar* (CYA).

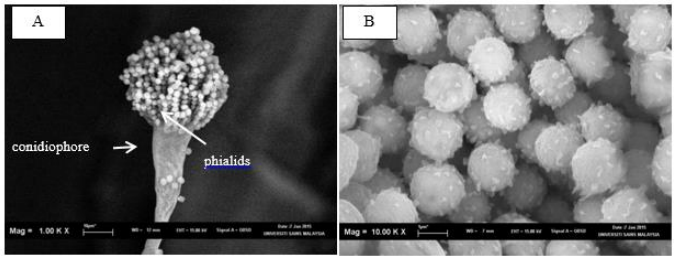

Figure 2b. Scanning electron micrographs of *A. fumigatus*; A) Conidiophore appears as wide, short and wrinkled surface with round vesicle and columnar head as well as uniseriate phialids (1000X); B) Globular spores in shape with spiny texture echinulate ornamentation (10,000 X).

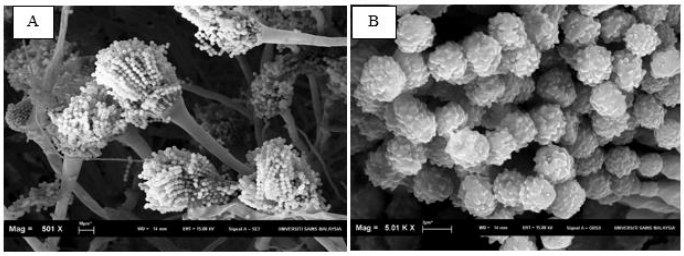

Figure 2c. Scanning electron micrographs of *Aspergillus* sp. strain no. 311; A) Conidiophore appears as wide, short and smooth surface with round vesicle and columnar head as well as uniseriate phialides (501X); B) s Globular spore in shape with distinctly wrinkled in texture and warty ornamentation (5010X).

ellipsoidal and sub-globular and spiny/distinctly wrinkled in the texture. The spore size was between 2.4 and 3.4 µm with average 2.9 µm (Figure 3b, Table 2). In comparison, long, wide and smooth conidiophore was detected for *A. sydowii,* with small vesicle, swollen at the base and biserate phialides. The spore size was between 3.3 and 4.1 µm (mean 3.7 µm) with globular and spiny texture (Figure 3c).

Table 2. Microscopic morphology of *Aspergillus* spp. isolated from clinical solid wastes.

Fungal species	Conidiophore*			Spore size (µm)**			Spore shape and surface***
	Structure	Vesicle head	Phialids	mean	max	min	
A. fumigatus	Wrinkled, wide/ short	round vesicle/ columnar head	uniseriate	2.4	2.9	1.9	Globular in shape with spiny texture echinulate ornamentation
Aspergillus sp. strain no. 311	Smooth, wide, short	round vesicle and columnar head	uniseriate	2.7	3.3	2.2	Globular in shape with distinctly wrinkled in texture and warty ornamentation
Aspergillus sp. strain no.145	long, narrow/ smooth	irregular vesicle and columnar head	biseriate, the metula was branched	2.9	3.4	2.4	Ellipsoidal and sub-globular with spiny/ distinctly wrinkled in texture
A. sydowii	long, narrow/ smooth	Small vesicle/ columnar head	biseriate	3.7	4.1	3.3	globular with spiny texture
A. parasiticus	long, narrow/ rough	round vesicle/ radiate head	uniseriate	3.9	5.0	3.4	Globular and sub-globular with smooth texture
A. flavus	long, narrow/ rough	round vesicle/ radiate head	biseriate	3.4	4.2	5	Globular to sub-globular spores with wrinkled and spiny texture, the spiny was arranged as parallel lines on the surface of spores
A. terreus var. terreus	smooth, narrow and long	round vesicle/ columnar head	biseriate	2.3	2.7	1.7	Globular and sub-globular with smooth/finely wrinkled in texture
A. caesiellus s	rough, narrow and long	round vesicle/ radiate/ columnar head	biseriate	28.2	51.3	41.6	Globular and spiny texture

*As shown using light microscope with 100X of magnification; **As determined using light microscope with 100X of magnification with Cell Sens Standard (CSS) programme (Version 1.4.1);***As shown using Scanning Electron Microscope (SEM).

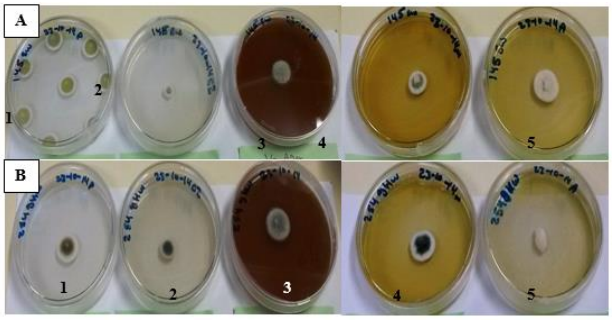

Figure 3a. Culture characteristics of *Aspergillus* sp. strain no. 145 isolated from clinical solid wastes collected from USM health center on different culture media. The isolates were incubated at 28˚C for 28 days. A) *Aspergillus* sp. strain no. 145SW; B) *A. sydowii*; 1) Potato Dextrose Agar (PDA); 2) Czapek-Dox Agar (CZ); 3) V8 juice agar (V8A); 4) Malt Extract Agar (MEA); 5) Czapek Yeast Extract Agar (CYA).

Figure 3b. Scanning electron micrographs of *Aspergillus* sp. strain no. 145; A) Conidiophore appears as long, narrow and smooth, with irregular vesicle and columnar head, biseriate phialids and branched metula (2040X); B) Ellipsoidal and sub-globular spores with spiny/ distinctly wrinkled in texture (5000X).

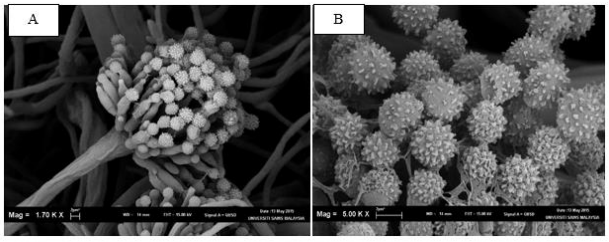

Figure 3c. Scanning electron micrographs of A. sydowii; A) Conidiophore appears as long, wide and smooth conidiophore, with small vesicle and swollen at the base and biseriate phialides (1700X); B) Globular spores with spiny texture (5000X).

A. flavus and *A. parasiticus* occurred as white edges colonies and dark green colour in the centre on V8A **(**Table 1**)**, while exhibited slight differences in their appearance on MEA, CZ and PDA, where *A. flavus* has an annular growth on PDA, olive and white on CZ and light green centre on the MEA, while, *A. parasiticus* colonies were white colour with dark green centre on MEA and light green colour on PDA and CZ (Figure 4a). In comparison, on CYA, *A. flavus* colonies were dark green colour with white edge, while *A. parasiticus* colonies occurred with white colour. The microscopic mor-

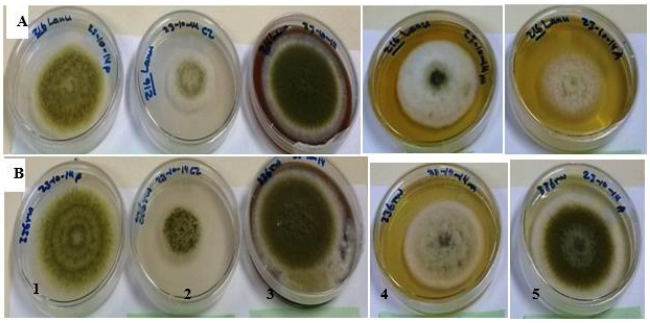

Figure 4a. Culture characteristics of *A. parasiticus* (A) and *A. flavus* (B). Both fungi were isolated from clinical solid wastes collected from USM health center on different culture media. The isolates were incubated at 28˚C for 7 days; 1) Potato Dextrose Agar (PDA); 2) Czapek-Dox Agar (CZ); 3) V8 juice agar (V8A); 4) Malt Extract Agar (MEA); 5) Czapek Yeast Extract Agar (CYA).

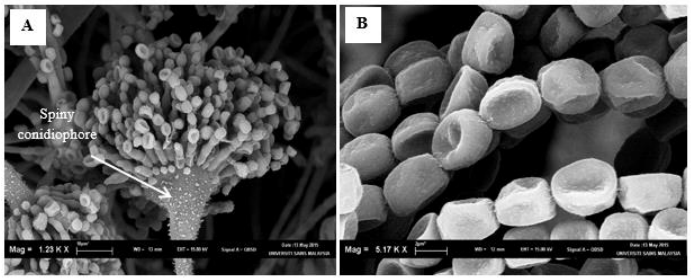

Figure 4b. Scanning electron micrographs of *A. parasiticus*; A) Conidiophore appears as short, wide and spiny with radiate vesicle and uniseriate phialides (1230X); B) Globular and sub-globular spores with smooth texture (5170X).

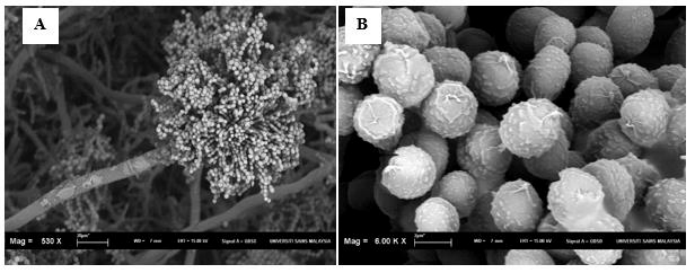

Figure 4c. Scanning electron micrographs of *A. flavus;* A*)* Conidiophore appears as long, narrow and rough with radiate vesicle and biseriate phialides (530X); B) Globular to sub-globular spores with wrinkled and spiny texture. The spiny is arranged as parallel lines on the surface of spore (6000X).

phologies observations showed that *A. parasiticus* has wide conidiophores with radiated vesicle and uniseriate phialides (Figure 4c), while *A. flavus* has long and narrow conidiophore with spiny texture, the spores arrangement was radiated with biseriate phialides (Figure 4b). Both strains have spore size ranged from 3.4 to 5 µm (mean 4.2 and 3.9 µm) for *A. flavus* and *A. parasiticus* respectively (Table 2). However, *A. Parasiticus* has a globular with smooth texture spores, while *A. flavus* spores were globular to sub-globular, wrinkled and spiny texture arranged as parallel lines on the surface of spore.

The cultured colonies of *A. terreus var. terreus* on different culture medium are presented in Figure 5 and Table 1. *A. terreus var. terreus* colony exhibited light yellow with white edges on the PDA medium. The colonies on CYA grown with brown colour in the centre and white edge. On the CZ the colonies were brown colour, sur-rounded by greenish colour and white edge. *A. terreus var. terreus* has a brown colony with white edge on MEA. *A. terreus var. terreus* has long, narrow and smooth conidio-phore structure with columnar head, round vesicle and biseriate phialides (Figure 5b). *A. terreus var. terreus* spores were globular and subglobular in shape with smooth/finely wrinkled in texture and the sizes ranged from 1.7 to 2.7 and 2.3 µm in average (Table 2).

Aspergillus caesiellus colonies on CYA and MEA appeared as white colonies, while the growth was white with green center on the PDA and CZ media**.** The fungal strain exhibited more growth on MEA and PDA compared to CZ and CYA (Figure6 a). *A. caesiellus* has rough, narrow and long conidiophore structure, with round vesicle and radiate head, sometime with columnar head with uniseriate phialides (Figure 6b). The spores were globular in shape with spiny texture with the size between 2.8 and 5.2 µm with an average of 4.2 µm (Table 1, Figure 6b).

The cultural and microscopic characteristics of *Aspergillus* spp. on different media represent a key parameters for the organization of fungi by phenotypic method (Diba, Kordbacheh, Mirhendi, Rezaie, & Mahmoudi, 2007; Guarro, Gené, & Stchigel, 1999). The phenotypic technique is a basic

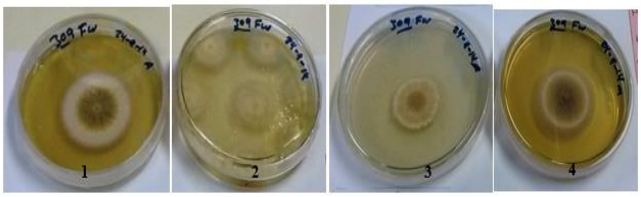

Figure 5a. Culture characteristics of *A. terreus var. terreus* isolated from clinical solid wastes collected from USM health center on different culture media. The isolates were incubated at 28˚C for 7 days; 1) Czapek Yeast Extract Agar (CYA); 2) Potato Dextrose Agar (PDA); 3) Czapek-Dox Agar (CZ); 4) Malt Extract Agar (MEA).

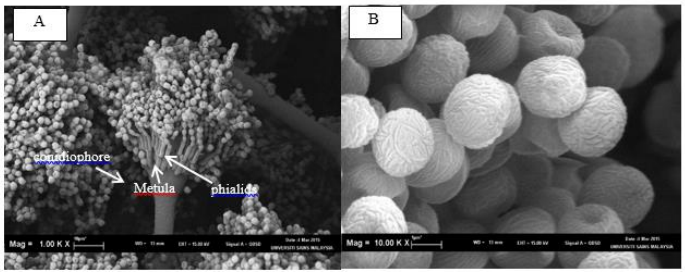

Figure 5b. Scanning electron micrographs of *A. terreus var. terreus;* A*)* Conidiophore appears as smooth, narrow and long with round vesicle and columnar head as well as biseriate phialides (1090X); B) Globular and sub-globular spores with smooth/finely wrinkled in texture (10,000X).

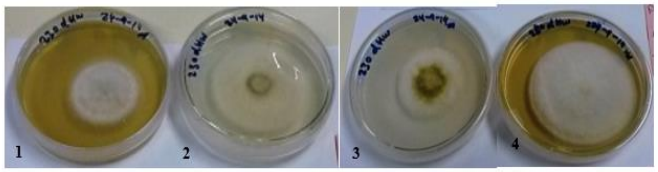

Figure 6a. Culture characteristics of *A. caesiellus* isolated from clinical solid wastes collected from USM health center on different culture media. The isolates were incubated at 28˚C for 7 days; 1) Czapek Yeast Extract Agar (CYA); 2) Potato Dextrose Agar (PDA); 3) Czapek-Dox Agar (CZ); 4) Malt Extract Agar (MEA).

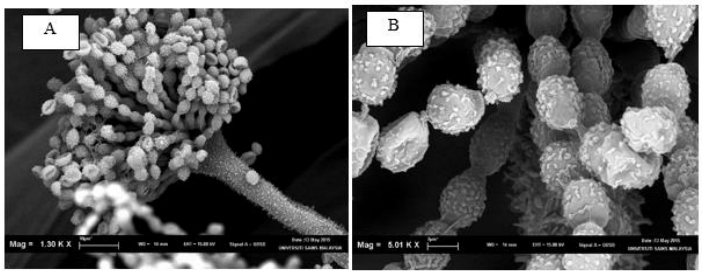

Figure 6b. Scanning electron micrographs of *A. caesiellus;* A*)* Conidiophore appears as long, narrow and spiny with round vesicle and radiate head as well as uniseriate phialides (1300X); B) Globular spores and spiny texture (5010X).

and effective tool for *Aspergillus* spp. identification. It has a high potential to give clear characteristics on culture media and under microscope. For the *Aspergillus* spp. the similarities among its species represent a critical step for the classification. American society for microbiology has mentioned that 89% of laboratories fungi are identified based on morphological characteristics (Diba *et al.*, 2007).

One of the limitations of using phenotypic method is the misconception about its value for the detection of some species such as *Aspergillus* spp. as noted in this study. Owing that, the SEM analyses for *Aspergillus* spp. should be conducted for determining the ultrastructure of spores and conidiophore. This technique has been used extensively for identifying many of the fungal species (Clarke & Griffiths, 1970; Eduard, Sandven, Johansen, & Bruun, 1985). Gao, Liu, and Yu (2007) identified two new strains of *A. flavus* (L and S strain) using the ultrastructure of spores and conidiophores. Moreover, *A. quitensis*, *A. amazonicus* and *A. Ecuadorensis* were classified as new strains based on the ultrastructure of spores and conidiophore as determined using SEM technique (Mares *et al.*, 2008). Zhang (2009) identified new *Aspergillus* species according to the ultrastructure of spores and conidiophore determined by SEM as well as based on morphological characteristics on different culture media. Meanwhile a study by Vestlund *et al.* (2014) had used SEM for identifying the bioaerosols with fungal spores. In this study, two strains identified as *Aspergillus* sp. strain no. 311and *Aspergillus* sp. strain no. 145 were determined as new strains obtained from the clinical wastes based on morphological analysis

In terms of the relationship between *Aspergillus* spp microstructures and clinical wastes it has been reported in the literature that the occurrence of fungi in the clinical wastes is depended on many factors such as pH, moisture and availability of nutrients**.** However, the nature of the clinical wastes as a plastic and fabric have more loading of fungi than those made up of metal and glasses (Noman *et al.*, 2016). The explanation for these differences is belongs to the surface properties of the solid wastes such as the ability to absorb humidity which play an important role in the adhesion of fungal spores. On contrast, Neely and Orloff (2001) revealed that the ability of fungi to survive in the clinical wastes relied on their surface structure. For instance, *Aspergillus* spp. survived more than *Fusarium* spp. because the *Aspergillus* spp. with rough and spiny spores enables it to adhere to the clinical waste samples. One more explanation for the investigation the microstructure of the fungal spores in the present study is the effect of spore structure and size on the transmission of the spores from one place to another. The fungal spores are transferred from the samples into the air by a mechanism of droplet adhesion. The process relied mainly on the spores' size, where the small spores are more easily to be suspended in the air than the large size spores (Baron, 2010; WHO, 2009). Therefore, spores of *Aspergillus* spp. are more frequencies in the air, since the size is ranging from 1.5 to less than 6 µm. These findings are in agree with previous work which revealed that the *A. fumigatus*, *Aspergillus* sp. strain no. 145 and *A. caesiellus* which have spiny spores were the most frequent in the clinical wastes (Noman *et al.*, 2016).

4. Conclusions

It can be concluded that the microstructures of the *Aspergillus* spp. spores play an important role in the contamination of clinical wastes, where the fungal spores with spiny surface have high adherence for the solid wastes. The SEM analysis was more useful for identifying the fine structure of the fungal isolates that belong to *Aspergillus* spp. and thus facilitate the identification process.

References

Barnett, H., & Hunter, B. (1998). *Illustrated genera of imperfect fungi; A comprehensive resource for recognizing, identifying, and learning various aspects of imperfect fungi* (4th ed.). St. Pau, MN: APS Press, The American Phytopathological Society.

Baron, P. (2010) *Generation and behavior of airborne particles (aerosols).* Retrieved from https://www.cdc.gov/niosh/topics/aerosols/pdfs/Aerosol_101.pdf?fbclid=IwAR0uFD71YA0gGb4w9VM2HShaEpeYRRzgDf_Xj2hL_trCnAThJBorHVEsZ30

Campbell, C., Johnson, E., & Warnock, D. (2013). *Identification of pathogenic fungi* (2nd ed.). Hoboken, NJ: John Wiley and Sons.

Clarke, J. H., & Griffiths, D. A. (1970). Ascospores of some common species of Eurotium (*Aspergillus glaucus*) as shown by scanning electron microscopy. *Transactions of the British Mycological Society*, *55*(1), 117-IN13.

Cole, G. T., & Samson, R. A. (1979). *Patterns of development in conidial fungi*. London, England: Pitman Publishing.

da Cunha, K. C., Sutton, D. A., Fothergill, A. W., Gené, J., Cano, J., Madrid, H., . . . Guarro, J. (2013). *In vitro* antifungal susceptibility and molecular identity of 99 clinical isolates of the opportunistic fungal genus *Curvularia***.** *Diagnostic Microbiology and Infectious Disease*, *76*(2), 168-174.

Diba, K., Kordbacheh, P., Mirhendi, S. H., Rezaie, S., & Mahmoudi, M. (2007). Identification of Aspergillus species using morphological characteristics. *Pakistan Journal of Medical Sciences*, *23*(6), 867.

Eduard, W**.**, Sandven, P**.** Johansen, B**.**, & Bruun, R. (1985). Identification and quantification of mould spores by scanning electron microscopy (SEM): Analysis of filter samples collected in Norwegian Saw Mills. *Proceedings of an International Symposium and Workshop on Lung Dosimetry Organised by the British Occupational Hygiene Society in Co-Operation with the Commission of the European Communities*, *1988*, 445–453.

Efaq, A. N., Rahman, N. N. N. A., Nagao, H., Al-Gheethi, A. A., Shahadat, M., & Kadir, M. A. (2015). Supercritical carbon dioxide as non-thermal alternative technology for safe handling of clinical wastes. *Environmental Processes*, *2*(4), 797-822.

Efaq, A. N., Al-Gheethi, A. A., Rahman, A., & Nagao, H. (2019). Morphological characteristics of black aspergilli isolated from clinical wastes. *Songklanakarin Journal of Science and Technology*, *41*(1), 181-191.

Ellis, M. B. (1971). Dematiaceous hyphomycetes. *Mycological Papers*, *125*, 1-30**.**

Gao, J., Liu, Z., & Yu, J. (2007). Identification of Aspergillus section Flavi in maize in northeastern China. *Mycopathologia*, *164*(2), 91-95.

Guarro, J., Gené, J., & Stchigel, A. M. (1999). Developments in fungal taxonomy. *Clinical Microbiology Reviews, 12*(3), 454-500.

Jensen, A. B., Aronstein, K., Flores, J. M., Vojvodic, S., Palacio, M. A., & Spivak, M. (2013). Standard methods for fungal brood disease research. *Journal of Apicultural Research*, *52*(1), 1-20.

Kumara, K. L., & Rawal, R. D. (2010). Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of Colletotrichum gloeosporioides causing anthracnose disease of papaya (Carrica papaya L). *Tropical Agricultural Research and Extension, 11*.

Mares, D., Andreotti, E., Maldonado, M. E., Pedrini, P., Colalongo, C., & Romagnoli, C. (2008). Three new species of Aspergillus from Amazonian forest soil (Ecuador). *Current Microbiology*, *57*(3), 222-229**.**

Nakada, M., Tanaka, C., Tsunewaki, K., & Tsuda, M. (1994). RFLP analysis for species separation in the genera Bipolaris and Curvularia. *Mycoscience*, *35*(3), 271-278.

Neely, A. N., & Orloff, M. M. (2001). Survival of some medically important fungi on hospital fabrics and plastics**.** *Journal of Clinical Microbiology*, *39*(9), 3360-3361.

Nielsen, K. F., Gravesen, S., Nielsen, P. A., Andersen, B., Thrane, U., & Frisvad, J. C. (1999). Production of mycotoxins on artificially and naturally infested

building materials. *Mycopathologia*, *145*(1), 43-56.

NMRC. (2015). Mycology online. Adelaide, Australia: National Mycology Reference Centre, the University of Adelaide. Retrieved from http://www.mycology.adelaide.edu.au

Noman, E. A., Al-Gheethi, A. A., Rahman, N. N. N. A., Nagao, H., & Kadir, M. A. (2016). Assessment of relevant fungal species in clinical solid wastes. *Environmental Science and Pollution Research, 23*(19), 19806-19824.

Noman, E. A., Al-Gheethi, A. A., Ab Rahman, N. N. N., Hideyuki, N., Talip, B. B. H. A., Mohamed, R. M. S. R., & Ab Kadir, M. O. (2018). Phenotypic identification of Penicillium spp. isolated from clinical wastes based on microstructure characteristics. *Malaysian Journal of Microbiology*, *14*(2), 88-95.

Promputtha, I., Jeewon, R., Lumyong, S., McKenzie, E. H., & Hyde, K. D. (2005). Ribosomal DNA fingerprinting in the identification of non sporulating endophytes from Magnolia liliifera (Magnoliaceae). *Fungal Diversity*.

Robert, A. S., János, V., & Christian, F. J. (2011). *Taxonomic studies on the genus Aspergillus-DTU Orbit, Studies in Mycology*. Utrecht, Netherlands: CBS-KNAW Fungal Biodiversity Centre. Retrieved from http://www.studiesinmycology.org/content/69/1.toc

Şahil, D., & Otag, F. (2013). Filamentous fungi isolated from clinical samples stored for a long time in the sand. *Clinic Microbial*, *2*(1), 1-2.

Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2010). Food and indoor fungi. Utrecht, Netherlands: CBS-KNAW Fungal Biodiversity Centre.

Samson, R. A., Noonim, P., Meijer, M., Houbraken, J. A. M. P., Frisvad, J. C., & Varga, J. (2007). Diagnostic tools to identify black aspergilli. *Studies in Mycology*, *59*, 129-145.

Silva, D. M., Batista, L. R., Rezende, E. F., Fungaro, M. H. P., Sartori, D., & Alves, E. (2011). Identification of fungi of the genus Aspergillus section Nigri using polyphasic taxonomy. *Brazilian Journal of Microbiology*, *42*(2), 761-773.

Sivanesan, A. (1987). Graminicolous species of Bipolaris, Curvularia, Drechslera, Exserohilum and their teleomorphs. Wallingford, England: CAB International.

Vestlund, A. T., Al-Ashaab, R., Tyrrel, S. F., Longhurst, P. J., Pollard, S. J., & Drew, G. H. (2014). Morphological classification of bioaerosols from composting using scanning electron microscopy. *Waste Management, 34*(7), 1101-1108.

Vieira, C. D., De Carvalho, M. A. R., De Resende, M. A., de Menezes Cussiol, N. A., Alvarez-Leite, M. E., Dos Santos, S. G., . . . de Macedo Farias, L. (2010). Isolation of clinically relevant fungal species from solid waste and environment of dental health services**.** *Letters in Applied Microbiology*, *51*(4), 370-376.

Watanabe, T. (2002). *Pictorial atlas of soil and seed fungi; Morphologies of cultured fungi and key to species* (2nd ed.). Boca Raton, FL: CRC Press.

World Health Organization. (2005). *Management of solid healthcare waste at primary healthcare centres. A decision making guide*. Geneva, Switzerland: Author.

World Health Organization. (2009). *Guidelines for indoor air quality: Dampness and mould.* Geneva, Switzerland: Author.

Zhang, Z. (2009). A new species of *Aspergillus***.** *International Journal of Biology*, *1*, 78-80.