Microscopic characterization of two black *Aspergillus, A. niger* and *A. aculeatus* from different substrates and indoor environment

Wardah Abdul Rahman and Latiffah Zakaria*

School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

Abstract

Black *Aspergillus* occurs in various types of substrates including indoor environment. In the present study, two species of black *Aspergillus*, *A. niger* and *A. aculeatus* were recovered from rice, groundnuts, spices, corn grains, agricultural soil and indoor environment. Both species were identified based on dark or black colony appearance and microscopic characteristics. Microscopic characteristics were observed using Scanning Electron Microscope, as well as light microscope. The characters to differentiate between *A. niger* and *A. aculeatus* were the formations of phialides and the shape and size of conidia. *Aspergillus niger* produced biseriate phialides and globose to subglobose conidia. As for *A. aculeatus*, the phialides were uniseriate, and the conidia were slightly brown, frequently ellipsoidal with echinulate and evenly spaced spines. Globose to subglobose conidia were also observed. Microscopic characterization is still important, not only to sort the isolates into their respective groups or sections, but it is relatively rapid identification to obtain faster results on the species identity

Keywords: black *Aspergillus*, *Aspergillus niger*, *Aspergillus aculeatus*, seriation, conidia

Article Info

Received 11th January 2020
Accepted 15th August 2020
Published 1st December 2020
*Corresponding author: Latiffah Zakaria; email: Lfah@usm.my

ISSN: 1823-7010, eISSN: 2600-7444

Introduction

Black *Aspergillus* spp. are among spoilage fungi contaminated food and feed, storage products as well as one of common fungi occur in indoor environment. Some species are beneficial for example there are strains of *A. niger* produced useful enzymes and organic acid such as lipases and citric acid used in food processing. Beneficial black *Aspergillus* spp. particularly *A. niger* are considered Generally Recognised as Safe (GRAS) by the United States Food and Drug Administration However, there are species of black *Aspergillus* that produced mycotoxin particularly ochratoxin A that contaminated cereal grains and species that are pathogens on plant and human [1]. Thus, it is important to conduct preliminary identification of black *Aspergillus* to assist in detail characterization of this group of aspergili.

Species of black *Aspergillus* are recognized based on the colony appearance which is often dark brown to black with black or near black conidia. These are the main features to sort the isolates of black *Aspergillus* and to distinguish the isolates of *Aspergillus* from other groups or sections of the genus *Aspergillus.* Morphological identification of *Aspergillus* is commonly based on microscopic characteristics of the philiades, conidial head, conidia and vesicle are important [2, 3]. It is relatively rapid method of identification to obtain faster identification results.

The present study was conducted to identify and characterize black *Aspergillus* from various sources, namely rice, groundnuts, spices, corn grains, agricultural soil and indoor environment using microscopic characteristics.

Materials and Methods

Morphological Identification

Results and Discussion

Microscopic characterization of black Aspergillus

A total of 168 isolates of black *Aspergillus* from rice, groundnuts, spices, corn grains, agricultural soil and indoor environment were recovered. Based on microscopic descriptions by [2, 3, 4] 144 isolates were identified as *A. niger* and 24 isolates as *A. aculeatus*.

Black *Aspergillus* spp. are easily identified based on their black to dark brown colonies. In a morphological study conducted by [6], the production of black or brown-black colonies was used for initial identification of *A. niger* from the other species of *Aspergillus*. In a study by [7], black *Aspergillus* were identified based on dark-coloured colonies. For microscopic characteristics, the phialides either uniseriate or biseriate are also commonly used for identification. Some species of black *Aspergillus* have been distinguished based on the phialides formation or seriation including *A. niger* and *A. aculeatus*. Efaq et al [8] reported that conidial ornamentation and conidiophores of black *Aspergillus* are important structures for morphological identification of black aspergilli species isolated from clinical wastes. Conidial ornamentation as well as the vesicle characteristics were also used to distinguish three black *Aspergillus* from beach sand [9].

Aspergillus niger

Aspergillus niger produced very dark brown to black conidial heads with irregularly-formed clear exudates (Figure 1A). The conidiophores were long, thick and smooth-walled (Figures 1B - C) with average width and length of 8-20 X 380-2300 µm. The phialides were biseriate with metulae covering the entire vesicle's surface (Figure 1C). The vesicles were large, globose (Figure 1D) to nearly spherical (Figure 1E) with average size of 30 - 80 µm wide. Conidia were globose to subglobose with occasionally finely rough (Figure 1F) to very rough with irregular ridges and bars (Figure 1G). Average size of conidia was 2.4 - 5.0 µm in diameter and occasionally arranged in chain.

For all the isolates of *A. niger* from rice, groundnuts, spices, corn grains, agricultural soil and indoor environment, the microscopic characteristics observed were similar. In the present study, the average width and length of conidiophores and vesicles observed were within the range of *A. niger* conidiophores and vesicles described by [2]. The shapes and textures of conidiophores, vesicle and conidia were similar with the descriptions by [2, 3, 4]. However, the conidial diameter (2.4 - 5.0 µm) was smaller than the size described by [2, 3, 4] of which the sizes were from 3.0 - 5.0 µm.

Aspergillus niger is one of the ubiquitous fungi found in the soil and subsequently can act as saprophyte. In indoor environment, *A. niger* is also among the prevalent indoor fungi and could easily found in any type of buildings. The findings of the present study were in accordance with previous reports in which *A. niger* was prevalent in many types of soil and indoor environment [19, 11, 12, 13, 7, 14]. The ability of the *A. niger* conidia to remain dormant in soil and air during unfavourable condition for growth might be one of the possible reasons. *Aspergillus niger* is also one of common spoilage fungi contaminated spices, ground nuts and corn grains [15, 16]. Thus, the conidia of *A. niger* is well designed for airborne dispersal and could grow when favorable environment are available [17].

Aspergillus aculeatus

Aspergillus aculeatus produced very dark brown to purple brown conidial heads (Figures 2A - B) and exudates were present (Figure 2B). The conidiphores were thick and smooth-walled (Figure 2C) with average width and length of 4 - 19 X 171 - 1150 µm. The phialides were uniseriate with metulae covering the entire vesicle's surface (Figure 2D). The vesicles were large, globose to elongate (Figure 2D) with average size of 20 - 68 µm wide. Conidia were slightly brown, globose to subglobose (Figure 2E). Ellipsoidal conidia with echinulate and evenly spaced spines were also frequently formed (Figure 2F). Average sizes of conidia were 2.6 - 5.3 µm in diameter and irregularly arranged in chain.

The distinguishing characters to differentiate between *A. niger* and *A. aculeatus* were the formations of phialides and the shape and size of conidia. In this study, *A. niger* produced biseriate phialides while *A. aculeatus* produced uniseriate phialides. According to [18, 19], phialides features can be used as one of the criteria to identify and characterize species of black *Aspergillus*, as biseriate species or subgroup showed morphological differences compared to uniseriate species. Previous studies have also shown that phialides formation and features were useful for preliminary identification and characterization of black *Aspergillus* from different sources or substrates such

as isolates from grapes [20], indoor environment [21], corn and soil [22]. Similar with *A. niger*, *A. aculeatus* is also common fungi found in food and feed [23] and in the soil [9]. *Aspergillus aculeatus* has also been reported to improve Cd tolerance and reduce Cd transportation in rice plant [24]

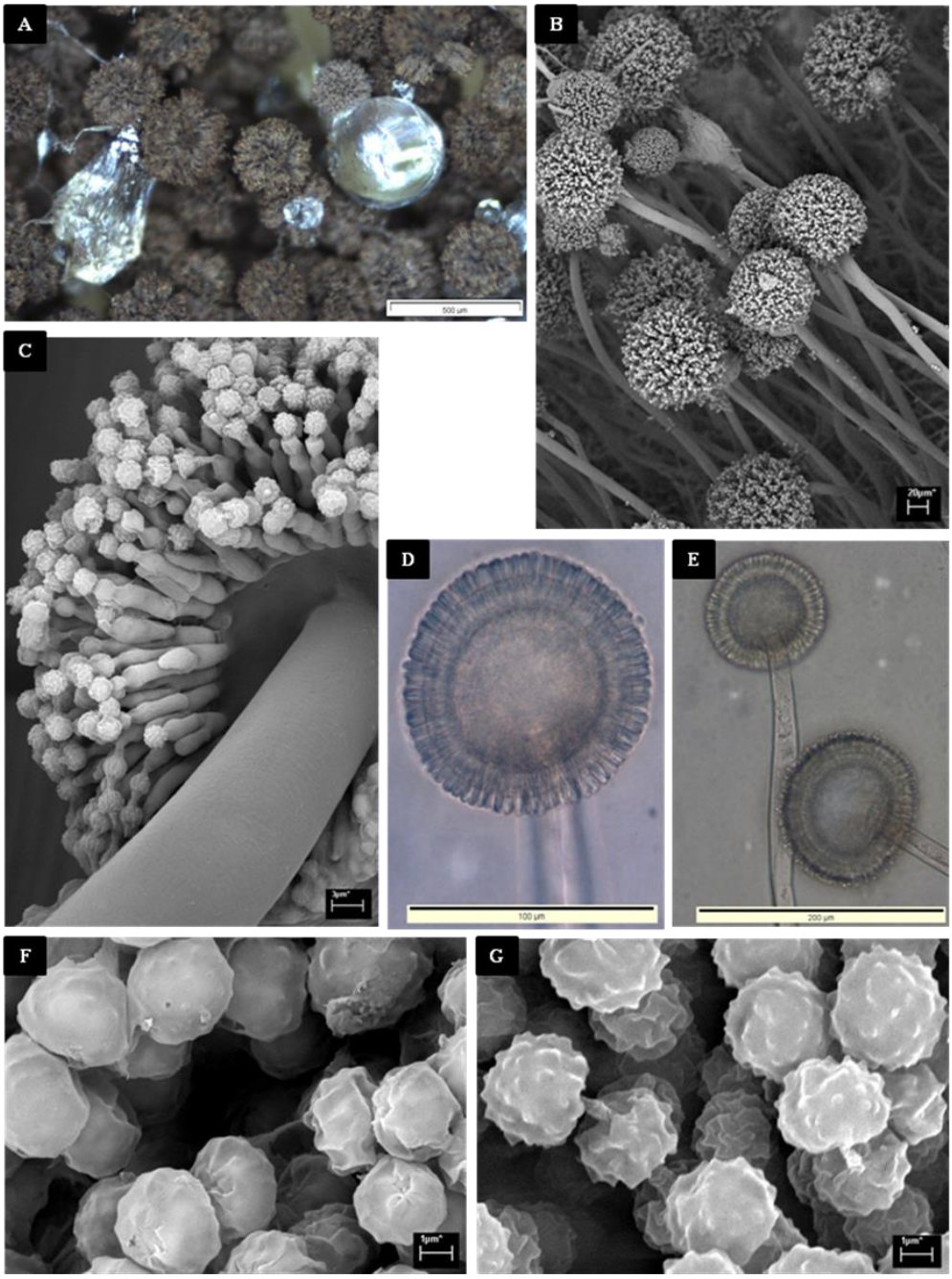

Figure 1. Microscopic characteristics of *A. niger*. (A) Very dark brown to black conidial heads; (B-C) Long, smooth-walled conidiophores (SEM); (D) Biseriate phialides with globose vesicle; (F) Biseriate phialides with nearly spherical vesicles; (F) Globose conidia with finely rough surface (SEM); (G) Subglobose conidia with very rough surface (SEM)

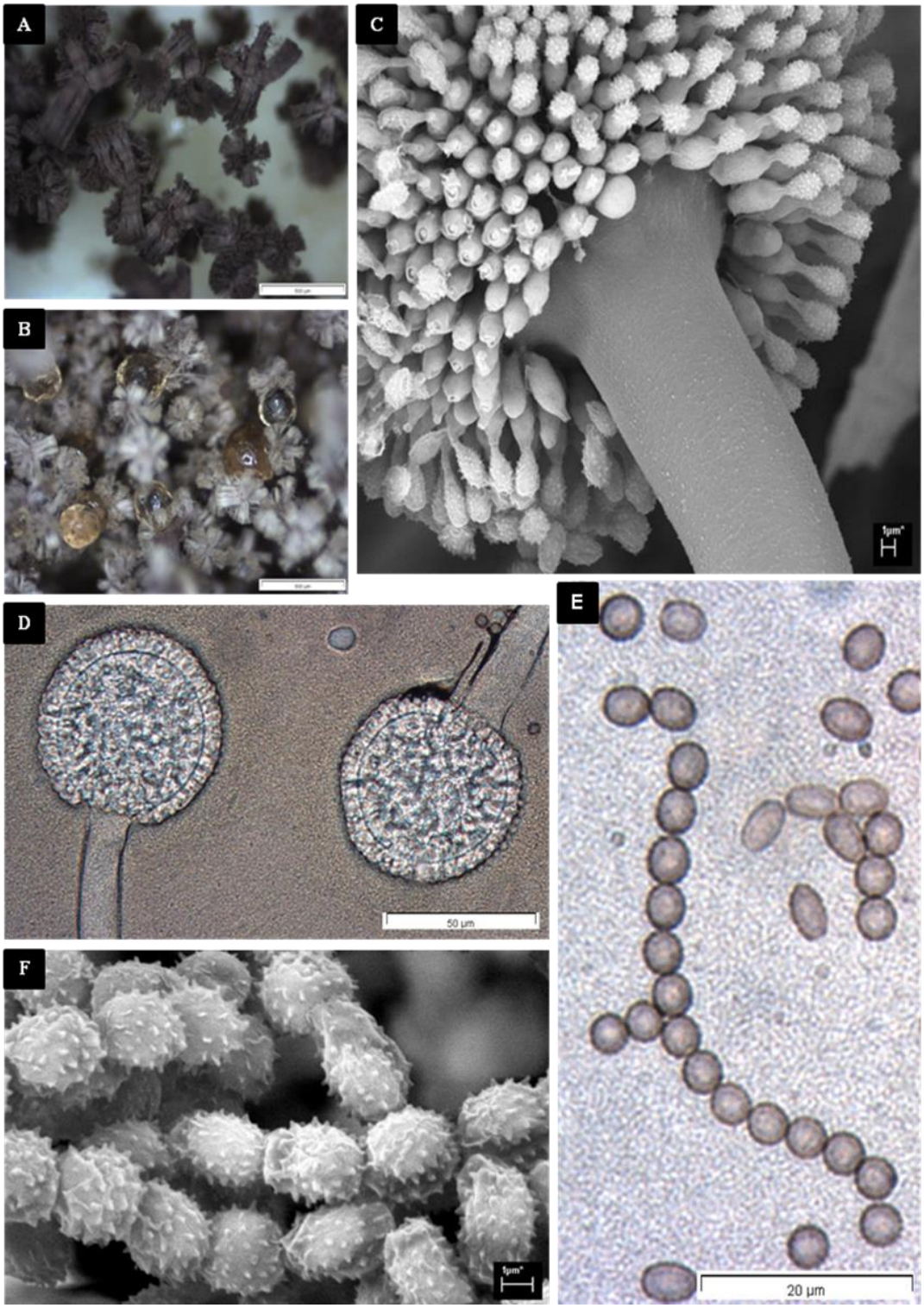

Figure 2. Microscopic characteristics of *A. aculeatus*. (A-B) Very dark brown to purple brown conidial heads; (C) Thick, smooth-walled conidiophores (SEM); (D) Uniseriate phialides; (E) Slightly brown globose to subglobose conidia; (F) Ellipsoidal conidia with echinulate surface (SEM)

Although, morphological characteristics of *A. aculeatus* are very similar and easily confused with another species of black *Aspergillus* which is *A. japonicus*, both species can be distinguish based on ellipsoidal conidia and wider size of vesicles (14-68 µm). In the present study, the formation of conidia and size of vesicles were used to identify the isolates of *A. aculeatus* from different substrates and to differentiate this species from *A. japonicus* which were similar with the descriptions by [2, 3, 25].

In conclusion, the phialides formation or seriation and conidial shape and size were used to distinguish between *A. niger* and *A. aculeatus* which can assist in morphological identification of both species of black *Aspergillus*.

Author Contributions

Both authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards

References

[1] Schuster, E., Dunn-Coleman, N., Frisvad, J.C. & van Dijck, P.W.M. (2002). On the safety of *Aspergillus niger*- a review. Applied Microbiology and Biotechnology 59: 426-435.
[2] Klich, M. A. (2002). Identification of common *Aspergillus* species. voor Schimmelcultures, Utrecht, The Netherlands.
[3] Pitt, J. I. & Hocking, A. D. (2009). *Fungi and Food Spoilage*. 3rd edition. (Springer Dordrecht) Heidelberg London New York.
[4] Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C. & Andersen, B. (2010). *Food and Indoor Fungi*. Utrecht, The Netherlands, CBS-KNAW Fungal Biodiversity Centre.
[5] Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B.-, Hubka, V., Klaassen, C. H. W., & Frisvad, J. C. (2014). Phylogeny, identification and nomenclature of the genus *Aspergillus.* Studies in Mycology 78 : 141–173.
[6] Mohammed, A. & Chala, A. (2014). Incidence of *Aspergillus* contamination of ground nut (*Arachis hypogaea*) in Eastern Ethiopia. African Journal of Microbiological Research 8 : 759–765.
[7] Zarei, F., Mirhendi, H., Motamedi, M., Ahmadi, B., Nouripour-Sisakht, S., Zarrinfar, H. & Hashemi, J. (2016). Black *Aspergillus* species isolated from clinical and environmental samples in Iran. Journal of Medical Microbiology 64 : 1454–1456.
[8] Efaq, A. N., Al-Gheethi, A. A., Nik Norulaini, N.A.B., Nagao, H. & Ab. Kadir M. O. (2019). Morphological characteristics of black aspergilli isolated from clinical wastes. Songklanakarin Journal of Science and Technology 41 (1) : 181-191.

[9] Teh, L.Y. & Latiffah, Z. (2018). Microscopic characteristics as preliminary identification of *Aspergillus* spp. from beach sand. Malaysian Journal of Microscopy 14 : 28-37.
[10] Leong, S. L., Hocking, A. D., & Scott, E. S. (2007). *Aspergillus* species producing ochratoxin A : isolation from vineyard soils and infection of Semillon bunches in Australia. Journal of Applied Microbiology 102 : 124–133.
[11] Ghosh, D., Priyanka, D., Das, A. K., & Uddin, N. (2011). Identification and distribution of aeromycoflora in the indoor environment of Shyambazar Metro-Railway Station, Kolkata, India. African Journal of Microbiology Research 5 : 5569–5574.
[12] Abdullah, S. K., & Muhammed, A. A. H. (2011). Prevalence of black aspergilli in soil at vineyards in Duhok, Kurdistan Region of Iraq. In: The 4th International Scientific Conference of Salahaddin University-Erbil, October 18-20, Iraq. pp. 966–969.
[13] Varga, J., Kocsube, S., Szigeti, G., Baranyi, N., Vagvlogyi, C., Despot, D. J., & Klaric, M. S. (2014). Occurrence of black Aspergilli in indoor environments of six countries. Toksikologiju-Archives of Industrial Hygiene and Toxicology 65 : 219–223.
[14] Douglas, S.I. & Robinson, V. K. (2018). Fungal Pollution of Indoor Air of Some Health Facilities in Rivers State. International Journal of Tropical Disease & Health 32(2): 1-7.
[15] Cumagun, C.J, Schwedt, B. & Karlovsky, P. (2018). Detection and quantification of aflatoxin-producing Aspergillus spp. in maize grain from the Philippines. In : Proceedings of the 40th Mycotoxin Workshop, Munich, Germany, 11–13 June 2018, pp. 90.
[16] Taniwaki, M.H., Pitt, J.I. & Magan, N. (2018). *Aspergillus* species and mycotoxins: occurrence and importance in major food commodities. Current Opinion in Food Science 23 : 38-43.
[17] Hayer, K., Stratford, M. & Archer, D. B. (2014). Germination of *Aspergillus niger* conidia is triggered by nitrogen compounds related to L-amino acids. Applied and Environmental Microbiology 80 : 6046–6053.
[18] Samson, R. A., Noonim, P., Meijer, M., Houbraken, J., Frisvad, J. C. & Varga, J. (2007). Diagnostic tools to identify black Aspergilli. Studies in Mycology 59 : 129-145.
[19] Noonim, P., Mahakarnchanakul, W., Varga, J., Frisvad, J. C. & Samson, R. A. (2008). Two novel species of *Aspergillus* section *Nigri* from Thai coffee beans. International Journal of Systematic and Evolutionary Microbiology 58 : 1727–1734.
[20] Serra, R., Caban, F. J., Perrone, G., & Castella, G. (2006). *Aspergillus ibericus* : a new species of section *Nigri* isolated from grapes. Mycologia 98 : 295–306.
[21] Jurjević, Ž., Peterson, S. W., Stea, G., Solfrizzo, M., Varga, J., Hubka, V. & Perrone, G. (2012). Two novel species of *Aspergillus* section *Nigri* from indoor air. IMA Fungus 3 : 159–173.
[22] Nyongesa, B. W., Okoth, S. & Ayugi, V. (2015). Identification key for *Aspergillus* species isolated from maize and soil of Nandi Country, Kenya. Advances in Microbiology 5 : 205–229.
[23] Perrone, G. & Gallo, A. (2017). *Aspergillus* species and their associated mycotoxins. Methods in Molecular Biology 1542: 33-49.
[24] Xie, Y., Li, X., Liu, X., Amombo, E., Chen, J. & Fu, J. (2018). Application of *Aspergillus aculeatus* to rice roots reduces Cd concentration in grain. Plant Soil 422 : 409–422.
[25] 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 : 761–773.