Endophytic *Aspergillus* species from corn kernels in Peninsular Malaysia

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Abstract. Endophytes are micro-organisms that infect and colonize internal tissues of host plants without causing obvious disease symptoms. Although most endophyte-plant relationships occur in the absence of the manifestation of disease, infections by some endophytic *Aspergillus* species may occur, leading to the production of mycotoxins in infected plant tissues by toxigenic species. In this study, endophytic *Aspergillus* species from kernels of corn plants in six states of Peninsular Malaysia were isolated and identified. A total of 178 isolates of endophytic *Aspergillus* belonging to two species, were recovered from surface disinfected corn kernels after 4-7 days of incubation on Potato Dextrose Agar (PDA), and identified using morphological characteristics on different growth media. Endophytic *Aspergillus flavus* was the most commonly isolated species (n=177), followed by *Aspergillus tubingensis* (n=1). Measures to control seed infection by endophytic *Aspergillus* species are required to improve corn seed health and preserve corn yield in Peninsular Malaysia.

1. Introduction
Endophytes are microbes that infect and colonize the below-surface tissues of healthy plant hosts without causing apparent symptoms of disease [1, 2]. Filamentous fungi in the genus *Aspergillus* have been reported to be associated with several crops such corn, peanut, and grape where their infections can cause maize kernel rot, peanut blight, and grape rot, respectively [3]. Although evidence suggests that some *Aspergillus* species colonize plant tissues as endophytes [4], very little is known about their occurrence as endophytes in tissues of corn plants in Peninsular Malaysia. Therefore, in the present study, isolation of endophytic *Aspergillus* from corn kernels was carried out and identified based on morphological characteristics.

2. Materials and Methods
About three to six mature healthy corn ears were collected from corn fields in several states in Peninsula Malaysia, namely Penang, Perak, Selangor, Negeri Sembilan, Terengganu, and Kelantan. The samples were transported in paper bags to the Plant Pathology Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang for further processing.

The endophytic *Aspergillus* were isolated using the modified protocol described by [5]. About 50 g of kernels were washed in running water, and surface sterilized by immersing in 70% ethanol for 30 seconds, 1% sodium hypochlorite solution for 1 min, and 95% ethanol for 3 minutes. Surface sterilized
kernels were rinsed in three changes of sterile distilled water, and blotted dry on sterile filter paper for 5 minutes. Surface sterilized kernels were plated on Potato Dextrose Agar (PDA) and incubated at 28°C for 4-7 days, and observed for the emergence of fungal mycelia. Prior to final plating of the samples, the efficacy of surface sterilization was confirmed using the imprint technique [6].

Identification of endophytic Aspergillus species was based on macroscopic and microscopic characteristics. Macroscopic characteristics were the colony colour, shape, elevation, texture, pigmentation, and growth rate on four differential media, namely PDA, Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYEA), and Dichloran 18% Glycerol Agar (DG18). Differential media are commonly used as qualitative methods for the tentative identification of Aspergillus species (Samson et al., 2010). Based on the growth pattern and sporulation of the isolates on the differential media, adequate morphological distinction between species can be observed [7]. For microscopic examination, the shape and size of conidia, as well as structure of hyphae and conidiogenous cells were observed. Identification was based on species descriptions by [8].

3. Results and Discussion
A total of 178 isolates of endophytic Aspergillus were isolated from the corn kernels. Based on morphological characteristics, two species were tentatively identified as Aspergillus flavus, and Aspergillus tubingensis. Endophytic A. flavus (n=177) was the most common species isolated compared to A. tubingensis (n=1).

Growth of the endophytic fungal isolates on different media showed variations in colony appearance, texture, and pigmentation. Colony appearance of A. flavus on PDA, MEA, CYEA, and DG18 (Figure 1) was similar with the colony descriptions of A. flavus by [9], and [10]. Microscopic examination of A. flavus revealed the presence of globose to sub-globose, smooth-walled conidia, borne on flask-shaped phialides. Phialides were held on rough-walled, subclavate vesicles, and mostly arranged in uniseriate, columnar, and radiate orientations around the vesicle head. The presence of subclavate vesicles, and the occurrence of both uniseriate and biseriate conidiogenous cells, were similar to descriptions of A. flavus by [11], and [12].

In similar studies by [13]-[15], A. flavus was reported as the most occurring fungal species in corn kernels. A. flavus is among fungal pathogens causing corn ear rot, the most serious disease of corn. Corn ear rot reduces kernel quality and affects crop yield. A. flavus is a mycotoxigenic fungus, producing
aflatoxins that contaminate corn kernels and their products, making them unhealthy for consumption by humans and animals. The dominance of endophytic *A. flavus* in corn kernels has been linked to its ability to most efficiently harness the dynamics of water activity within the corn kernels as influenced by factors such as duration of hybrid maturity, host genotype, and air temperature and humidity during the growth season, for its growth and development, compared to other fungi [9].

Colony appearance of *A. tubingensis* on PDA, MEA, CYEA, and DG18 (Figure 2) was similar with the descriptions of *A. tubingensis* by [16], [17]. Microscopic examination of *A. tubingensis* cultures revealed the presence of brown to black, globose, and rough-walled conidia, borne on flask-shaped uniseriate phialides, and supported by large globose vesicles arranged in a radiate orientation around the vesicle head. The shapes of conidia and vesicles, and seriation of phialides, were also similar to the descriptions of [16].

![Figure 2](image-url)

*Figure 2*. Macromorphology of *A. tubingensis* on PDA and MEA. Colony appearance (A) and pigmentation (B) of *A. tubingensis* on PDA; colony appearance (C) and Pigmentation (D) of *A. tubingensis* on MEA.

Previous studies reported the occurrence of endophytic *A. tubingensis* in corn kernels in Mid-Western USA. Although very little is known about the pathogenicity and mycotoxicity of *A. tubingensis* in infected corn tissues, a reduction in seed germination and seedling growth in seeds inoculated with conidia of *A. tubingensis* [17], [18].

4. Conclusion
The study showed that although *A. flavus* and *A. tubingensis* were the endophytic *Aspergillus* species colonizing corn kernels in Peninsular Malaysia, *A. flavus* was predominant. *A. flavus* is a known causative agent of corn ear rot, and a producer of aflatoxins in corn kernels, while *A. tubingensis* has been associated with poor seed germination and seedling growth. Measures to control seed infection by endophytic *Aspergillus* species are required to improve corn seed health and preserve yield.

References
[1] Battilani P, Barbano C and Piva G 2008 *World Mycotoxin J.* 1 449-456.
[2] Carroll G C 1986 The biology of endophytism in plants with particular reference to woody plants. In: Fokkema N J and van den Heuvel J. (eds.). Microbiology of the phyllosphere. Cambridge (UK): Cambridge University Press. p 205–222.
[3] Giorni P, Magan N and Battilani P 2009 *Int. J. Food Microbiol.* **130** 213-218.
[4] Goutam J, Singh S, Kharwar R N and Ramaraj V 2016 *Biol. Med. (Aligarh)* **8** (7) 1000349.
[5] Jackson-Ziems T A, Giesler L J, Harveson R M, Korus K A, Liu B and Wegulo S N 2012 University of Nebraska, Lincoln. Retrieved from http://extensionpublications.unl.edu/assets/pdf/ec1901.pdf Accessed on: 18/09/2020.
[6] Lahouar A, Marin S, Crespo-Sempere A, Said S and Sanchis V 2016 *Rev. Argent. Microbiol.* **48** 78-85.
[7] Mangal M, Bansal S and Sharma M 2014 *Legume Res.* **37** (4) 372-378.
[8] Meijer M, Houbraken J A M P, Dalhuijsen S, Samson R A and De Vries R P 2011 *Studies in Mycology* **69** 19-30.
[9] Ojiambó P S, Battilani P, Cary J W, Blum B H and Carbone I 2018 *Phytopathology* **108** 1024-37.
[10] Palencia E R 2006 Endophytic associations of species in the *Aspergillus* Section *Nigri* with Maize (*Zea mays*) and peanut (*Arachis hypogea*) hosts, and their mycotoxins. Ph.D. Dissertation, University of Georgia, Athens. pp. 1-186.
[11] Samson R A, Houbraken J, Thrane U, Frisvad J C and Andersen B 2010 Food and Indoor Fungi, Second Edition. Utrecht, The Netherlands: CBS-KNAW Fungal Biodiversity Centre. pp. 1-390.
[12] Schulz B, Wanke U, Draeger S and Aust H J 1993 *Mycol Res.* **97** 1447–50.
[13] Simoes M F, Santos C and Lima N 2013 *Microscopy and Microanalysis* **19** (5) 1151-58.
[14] St-Germain G and Summerbell R 1996 Identifying filamentous fungi – A clinical laboratory handbook, 1st Edition. Belmont, California: Star Publishing Co. p. 314.
[15] Weieneth L K 2015 Seedborne Black Aspergillus species as maize seedling pathogens: role of fumonisins production and interaction with soilborne Pythium species. M.Sc. Thesis, Department of Plant Pathology and Microbiology, Iowa State University. pp. 1-66.
[16] Wilson D 1995 *Oikos.* **73** 274-276.
[17] World Health Organisation 2018 Aflatoxins. Food Safety Digest, Department of Food safety and Zoonoses. REF. No.: WHO/NHM/FOS/RAM/18.1. 2018. Available at: https://www.who.int/foodsafety/FSDigest_Aflatoxins_EN.pdf. Accessed 18/09/2020.
[18] Zakaria L and Ning C H 2013 *Trop Life Sci Res.* **24** 85-90.