



Aspergillus species isolated from mangrove forests in Borneo Island, Sarawak, Malaysia

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The occurrence of *Aspergillus* is worldwide but mainly in tropical and subtropical regions (Domsch et al. 1980; Christensen & Tuthill 1985). *Aspergillus* spp. are isolated from soil, sea water, plant debris, compost and litter (Raper & Fennell 1965). *Aspergillus* spp. are able to develop into many new strains through the process of heterokaryosis or parasexual reproduction and result high diversity in the environment. (Raper & Fennell, 1977; Papagianni 2004). This study aims to project the diversity of *Aspergillus* spp. on mangrove soil and leaf litters of four mangrove locations in Sarawak.

Materials and Methods

Sample collection and fungal isolation: Mangrove soil and 100–200 g of dried leaf litters were collected at random from four locations, i.e. Sematan, Lundu, Kampung Bako, Bako in the Borneo Island of Sarawak. Soil samples were pretreated following Warcup (1950). Five replicates were made for different soil samples and incubated at 28°C for seven days. Dried leaf litter samples were sliced into 1cm pieces, directly plated onto Malt Extract Agar (MEA) and then incubated at 28°C for seven days.

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Identification: For identification, the fungi isolates were grown on five different media such as Czapek Yeast Extract Agar (incubated at 25°C (CYA25), CYA37 (incubated at 37°C), CY20S (added with 20% sucrose incubated at 25°C), Malt Extract Agar (MEA) and Czapek Dox solution agar (CZ) (incubated at 25°C). For each culture, five plates were used in triplicates. Each plate was inoculated at three points, equidistant from the center and incubated in the dark for seven days. The strains were identified using current universal keys of identification (Raper & Fennell 1977; Pitt 1979; Domsch et al. 1980; Klich 2002). Notes of IMI description were also used for identification.

Microscopic Observation

A small tuft of mycelium and conidiophores were lifted from a fairly young portion of the colony, placed in a drop of alcohol on a glass slide and gently teased out. A drop of lactophenol blue or acid fuchsin was used to stain. The appearance of foot cell, conidiophores, presence of metulae (sterigmata) and conidia were observed. Digital photographs were taken by a Nikon camera attached to Leica Microscope.

Result and Discussion

Fungal Isolation: A total of 138 isolates belonging to five species of *Aspergillus* were isolated from the mangrove soil and leaf litter samples, collected from different sampling sites in four mangrove forests in Sarawak. Based on both macroscopic and microscopic observations, using an identification key, individual isolates were classified within the genus *Aspergillus*, belonging to three subgenera, four section and five species. The fungal isolates were identified as *A. terreus* Thom, *A. flavipes* Bainier and Sartory, *A. carneus* Blochwitz, *A. fumigatus* Fresenius and *A. clavatus* Desmazieres. The most frequently isolated species was *A. flavipes* (63.04%), followed by *A. fumigatus* (16.7%), *A. terreus* (13.04%), *A. carneus* (5.8%) and *A. clavatus* (1.44%). The total isolates of *Aspergillus* from mangrove soil and leaf litters in Sarawak shown in Figure 1.

Morphological Characteristics

All the five isolates showed significant growth rate level ($P < 0.05$) on the media tested as in Table 1. All the five species isolated were varying in texture, vesicle shape, pigment production and sporulation (Images 1-5).

Klich (2002) reported nearly 200 species of *Aspergillus* primarily from India, Europe, Egypt, Syria, tropical Africa, Japan and North America. Since there is no record for Borneo Island, this work has documented five different species of *Aspergillus* from the mangrove soils and leaf litters. From this preliminary observation, *A. fumigatus* Fresenius, *A. flavipes* Bainier and Sartory, *A. carneus* Blochwitz and *A. clavatus* Desmazieres, were morphologically more variable in conidial ornamentation, phialide shapes and pigmentation than described by Raper & Fennell (1965). In this study, *A. clavatus* was found interesting because of its yellow pigment and long conidiophores ranging from 3–5 cm on the leaf litters found in Kampung Bako while the other isolate of *A. clavatus* from Sematan, had short conidiophores ranging from 2.5–3.5 cm. Besides that, *A. carneus* showed reddish sclerotia on MEA at room temperature. This is an important character of this species.

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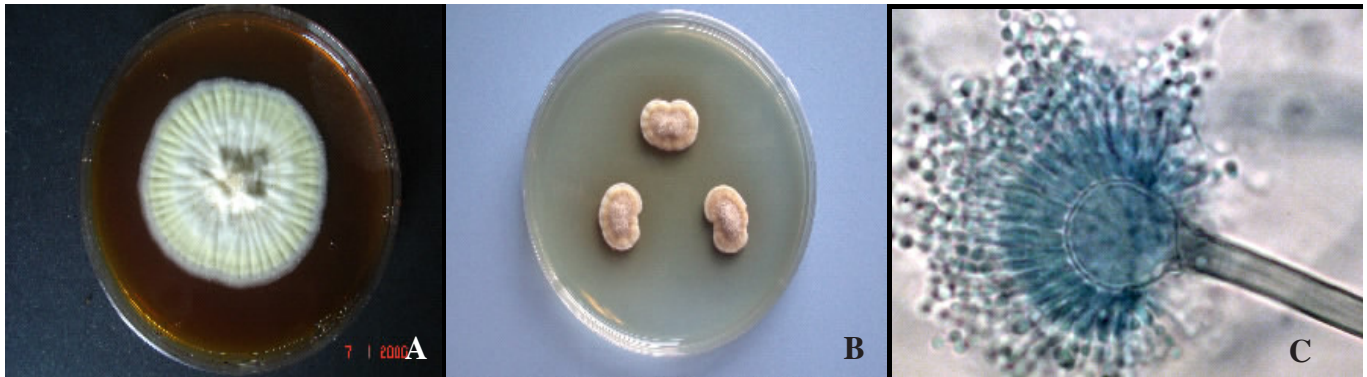


Image 1. (A-B) Colonies of *A. flavipes* on CYA and MEA at 25°C. (C) Vesicle shape subglobose; biseriate x 40; long phialides; spatulate to subglobose vesicles; conidia varies from globose and smooth walled



Image 2. (A-B) *Aspergillus terreus* on PDA and CYA at 25°C; Colony surface- white mycelia with sandy brown spores. (C) Conidial heads- splitting into divergent column; Vesicles suglobose with biseriate sterigmata x 100.

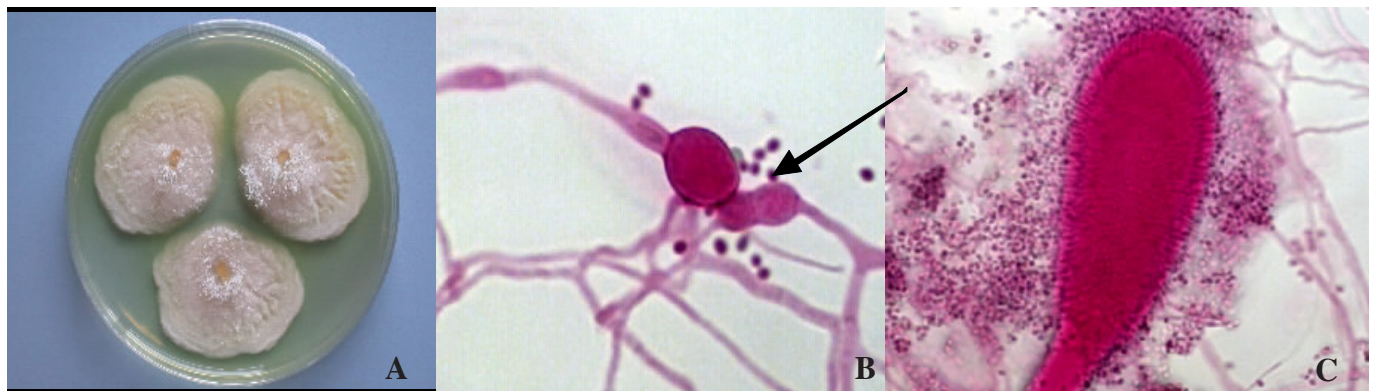


Image 3. (A) *Aspergillus clavatus* on MEA at 25°C produced yellow pigment; (c) swollen hyphal structures (b) bearing long conidiophores (3-5 cm); clavate shaped vesicles; conidia smooth walled, ellipsoidal x 100.

Table 1. Average growth rate of *Aspergillus* species on different media

Species name	Average growth rate (cm/day)			
	MEA	CYA	CY20S	CZ
<i>Aspergillus flavipes</i>	0.55 ± 0.03c	0.76 ± 0.04d	0.28 ± 0.05a	0.32 ± 0.02ab
<i>Aspergillus fumigatus</i>	0.80 ± 1.00d	0.73 ± 0.05c	0.53 ± 0.03a	0.64 ± 0.02b
<i>Aspergillus terreus</i>	0.45 ± 0.01b	0.48 ± 0.01ab	0.37 ± 0.01a	0.47 ± 0.01b
<i>Aspergillus carneus</i>	0.71 ± 0.06b	0.73 ± 0.02b	0.36 ± 0.01a	0.75 ± 0.02b
<i>Aspergillus clavatus</i>	0.84 ± 0.00d	0.78 ± 0.02c	0.46 ± 0.04a	0.55 ± 0.03b

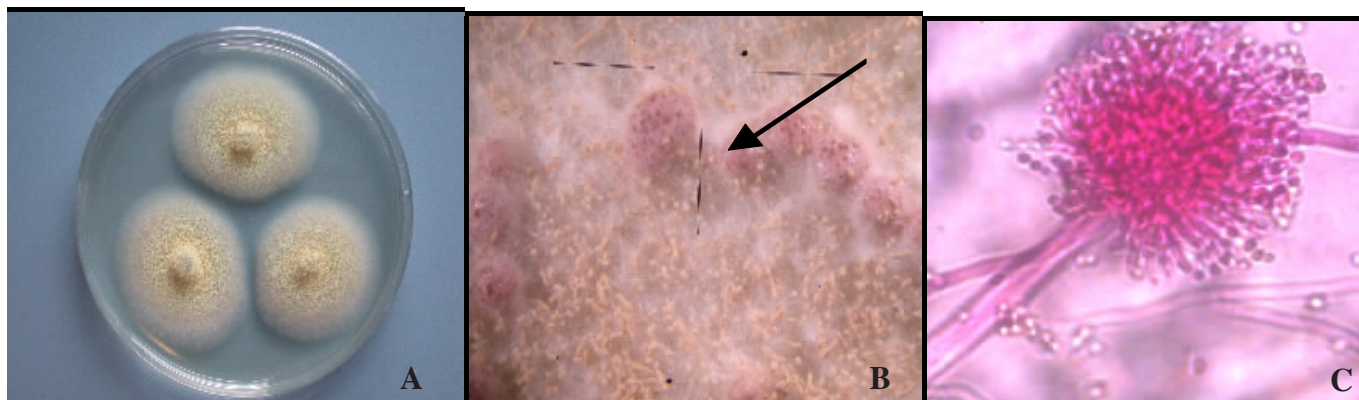


Image 4. (A) *Aspergillus carneus* on CYA and MEA are similar (B) reddish sclerotia on MEA only (C) subglobose vesicles; biseriata; conidia smooth walled and subglobose to globose x100.

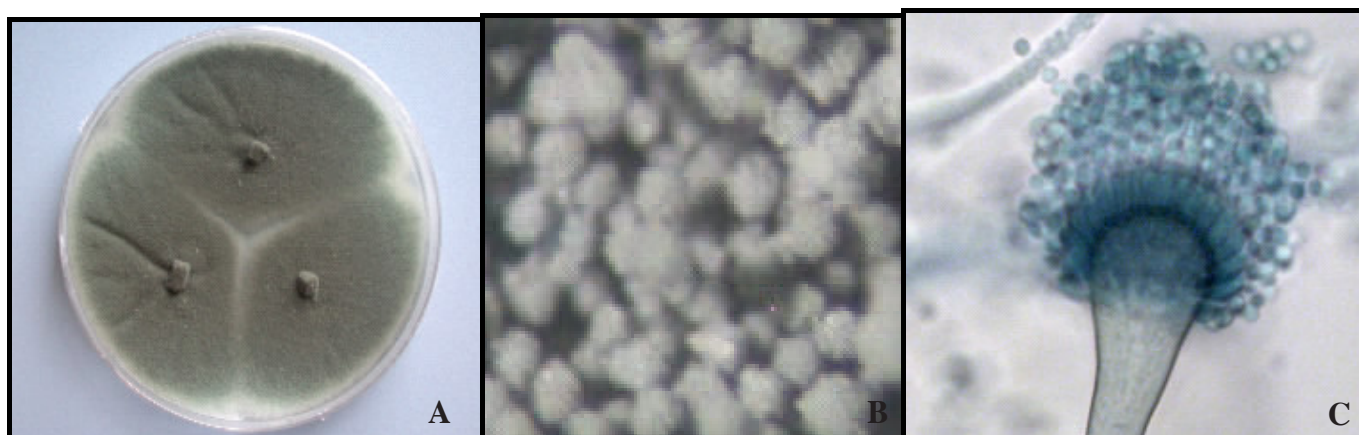


Image 5. (A) *Aspergillus fumigatus* on CYA at 25°C (B) spores white to dark greenish in color (C) radiate shaped vesicles; uniseriate; conidia smooth and subglobose in shape x100.

Conclusion

In conclusion, five species of *Aspergillus* were obtained i.e. *A. fumigatus* Fresenius, *A. flavipes* Bainier and Sartory, *A. carneus* Blochwitz and *A. clavatus* Desmazieres are reported from mangrove soil and leaf litters at Kampung Bako, Bako, Lundu and Sematan, Sarawak. Morphological characteristics of these five species were distinct. Colony growth characteristics were media dependent and were different for each species

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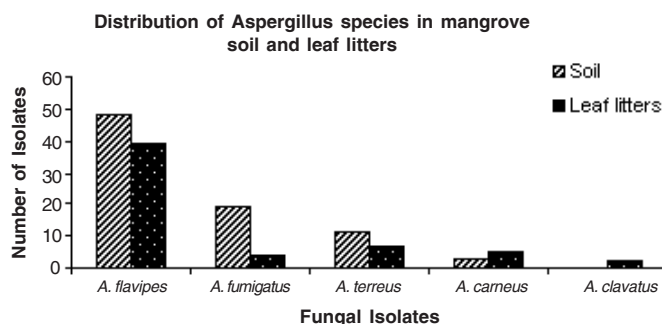


Figure 1. Total number of *Aspergillus* spp. isolated from mangrove soil and leaf litters

