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 parascusal reproduction and result high diversity in the environment. (Raper & Fennell, 1977; Papagian 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.

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

<table>
<thead>
<tr>
<th>Species name</th>
<th>MEA</th>
<th>CYA</th>
<th>CY20S</th>
<th>CZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavipes</td>
<td>0.55 ± 0.03c</td>
<td>0.76 ± 0.04d</td>
<td>0.28 ± 0.05a</td>
<td>0.32 ± 0.02ab</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>0.80 ± 1.00d</td>
<td>0.73 ± 0.05c</td>
<td>0.53 ± 0.03a</td>
<td>0.64 ± 0.02b</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>0.45 ± 0.01b</td>
<td>0.48 ± 0.01ab</td>
<td>0.37 ± 0.01a</td>
<td>0.47 ± 0.01b</td>
</tr>
<tr>
<td>Aspergillus carneus</td>
<td>0.71 ± 0.06b</td>
<td>0.73 ± 0.02b</td>
<td>0.36 ± 0.01a</td>
<td>0.75 ± 0.02b</td>
</tr>
<tr>
<td>Aspergillus clavatus</td>
<td>0.84 ± 0.00d</td>
<td>0.78 ± 0.02c</td>
<td>0.46 ± 0.04a</td>
<td>0.55 ± 0.03b</td>
</tr>
</tbody>
</table>

Image 1. (A-B) Colonies of A. flavipes on CYA and MEA at 25°C. (C) Vesicle shape subglobose; biseriate x 40; long phialides; spathulate 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.
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Image 4. (A) Aspergillus carneus on CYA and MEA are similar (B) reddish sclerotia on MEA only (C) subglobose vesicles; biseriate; conidia smooth walled and subglobose to globose x100.

Aspergillus carneus

Aspergillus fumigatus

Conclusion

In conclusion, five species of Aspergillus were obtained i.e. A. fumigatus Fresenius, A. flavipes Bainier and Saritory, A. carneus Blochwitz and A. clavatus Desmazeres 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.

REFERENCES


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

Distribution of Aspergillus species in mangrove soil and leaf litters

Fungal Isolates

Aspergillus carneus

Aspergillus clavatus

Aspergillus terreus

Aspergillus fumigatus

Aspergillus flavipes

Distribution of Aspergillus species in mangrove soil and leaf litters

Sol

Leaf litters

Number of Isolates

0

10

20

30

40

50

60

A. flavipes

A. fumigatus

A. terreus

A. carneus

A. clavatus

A. flavipes

A. fumigatus

A. terreus

A. carneus

A. clavatus

Distribution of Aspergillus species in mangrove soil and leaf litters

Number of Isolates

0

10

20

30

40

50

60

A. flavipes

A. fumigatus

A. terreus

A. carneus

A. clavatus

A. flavipes

A. fumigatus

A. terreus

A. carneus

A. clavatus