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Ohoratoxin producing *Aspergillus* spp. isolated from tropical soils in Sarawak, Malaysia

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Aspergillus spp. has been widely studied for mycotoxin analysis. Mycotoxin is considered one of the chemical groups that causes serious side effects in humans and animals. Although Aspergillus spp. contains bioactive compounds, there is a need to screen for mycotoxin metabolites since mycotoxin is hazardous to human health. Ochratoxin is a mycotoxin with nephrotoxic, nephrocarcinogenic, teratogenic and immunosuppressive properties, and has received growing interest in the scientific community and food committees in the last few years (Battaglia et al. 1996; Abarca et al. 2003). Only species belonging to the genera Aspergillus and Penicillium have been reported as capable of producing They were initially described by Scott (1965) in Aspergillus ochraceus but have also been found in other species of the section Circumdati: A. alliaceus, A. melleus, A. ostianus, A. petrakii, A. sclerotiorum, A.

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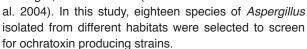
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sulphureus (Hesseltine et al. 1972), A. albertensis, A. auricomus (Varga et al. 1996); as well as in the black aspergilli of section Nigri: A. niger var. niger, A. carbonarius (Samson et



Material and Methods

Fungal isolates: Eighteen isolates of Aspergillus species were selected (Table 1) and grown on potato dextrose agar (PDA), Czapek's yeast extract agar (CYA), and Malt extract agar (MEA), incubated for 7 days at 25°C. The strains were identified based on Raper & Fennell (1965) and Klich (2002). Colonies were mounted in lactophenol blue and images were taken by using a NIKON digital camera.

Extraction and immunochemical tests by using ELISA kit: Each fungus was cultured in a Czapek's yeast extract broth (CYB) medium for 7 days and incubated at 25°C, in shaker of 120rpm. Crude fermentation broth was blended thoroughly and centrifuged at 4000rpm for 5 minutes. The supernatant was passed through a filtration membrane (0.22µm, Minipore). Then the homogenized broth was extracted with chloroform. The combined organic extract was evaporated under reduced pressure yielding a crude semi-solid (Wang 2002). Then, the extracts were tested by using enzyme-linked immunosorbent assays (ELISAs) test for positive results. ELISA positives were confirmed by HPLC technique.

OA detection by HPLC: The samples were analysed by using a reverse phase HPLC equipped with a Jasco FP-920 fluorescence detector (330nm excitation wavelength, 460nm emission wavelength). Chromatographic separations were performed on an analytical column (symmetry waters C18 ODS2, 150mm x 3.9mm, 5µm) fitted with a precolumn with the same stationary phase. The mobile phase used was pumped at 1.0ml/min and consisted of an isocratic program as follows: acetonitrile/ water/acetic acid (99:99:2, v/v). The injection volume was 20μl. Samples were taken as positive for OA presence if they yielded a peak at a retention time similar to the OA standard peak (approximately 4 min), with a height five times higher than the baseline noise.

Results and Discussion

ELISAs test: Altogether, 18 Aspergillus strains were tested for OA production by the immunochemical test. Among these, only two strains of Aspergillus, namely, A. carbonarius and A. sulphureus produced OA when tested with ELISA. The other sixteen strains of Aspergillus did not produce this toxin (Table 1). In temperate regions, A. carbonarius and A. sulphureus have been widely