Ohcratoxin 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 ochratoxins. 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. 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 al. 2004). In this study, eighteen species of *Aspergillus* isolated from different habitats were selected to screen for ochratoxin producing strains.

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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