SPECIES SPECIFIC PROFILES OF SECONDARY METABOLITES WITHIN THE GENUS FUSARIUM, OBTAINED BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

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SUMMARY

Extracts of pure cultures of Fusarium species were analysed by reversed phase High Performance Liquid Chromatography. The peaks recorded by the Photodiode-Array UV-VIS detector were characterized by their alkylphenone retention time index, and by their UV-VIS spectrum (200-600 nm). The number of biosynthetic families (chemosyndromes) was counted by grouping the peaks having similar UV-VIS spectra.

All the investigated Fusarium species had a specific profile of 4-13 chemosyndromes, of which some were species specific while others could be recorded in more than one species.

INTRODUCTION

The main characters used in Fusarium taxonomy is the morphology of the conidia and the conidiogenous cells, while growth rates (colony diameters) and pigmentation are used as secondary criteria (1,2,3,4). It has been demonstrated that profiles of secondary metabolites (including mycotoxins) are closely linked to species within the genera of Penicillium and Aspergillus (5). Due to misidentifications and the use of insufficient taxonomic systems, the connection between the profiles of secondary metabolites and Fusarium species is marked by confusion (6). Using a simple Thin-Layer Chromatography (TLC) method Fusarium isolates can be screened for production of mycotoxins and other secondary
metabolites (7). However, this method yields a species specific pattern of known and unknown metabolites. More informations are obtained using analyses of fungal extracts by High Performance Liquid Chromatography (HPLC) with Photodiode-Array detection, as the chromatographic peaks are characterized by their UV-VIS spectrum and an alkylphenone index (8).

MATERIALS AND METHODS

*Fusarium* cultures were grown on YES (yeast extract sucrose) agar for 14 days at 25°C. The cultures were extracted with chloroform/methanol (2/1) followed by ethyl acetate/acetone (1/1). The combined organic phase were concentrated, defatted, and filtered before injection into the chromatograph (Hewlett Packard HP 1090M). The analyses were performed on a Nucleosil-5µm C18 column, using a gradient solvent system (water/acetonitrile). The alkylphenone index (RI) were calculated as the retention time of the metabolite relative to the retention times of 7 homologous alkylphenones. Details concerning sample preparation, chromatographic parameters, and the alkylphenone indices are reported elsewhere (8).

RESULTS AND DISCUSSION

A direct comparison of HPLC chromatograms shows that each *Fusarium* species has a specific pattern of peaks. The patterns consist of peaks at specific retention indices (RI) and relative peak heights. Different isolates show the same pattern of peaks both with respect to retention index and peak height ratios. Closely related species, according to the traditional taxonomy, may have quite different peak patterns. This can be demonstrated by chromatograms of *F. tricinctum*, *F. poae*, and *F. sporotrichioides* (Fig. 1), which earlier have been merged into one species (9).

The use of a photodiode-array detector allows the UV-VIS spectrum (200-600 nm) of each peak to be recorded during the HPLC run. Using this facility it is possible to distinguish between