Centraalbureau voor Schimmelcultures
Fungal Biodiversity Centre
Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW)

Report

Aspergillus niger DS47447 (EPO712-9) and Fermentation broth JLL 03 006 IDF C1 Filtrated UF concentrate JLL 03 066 IDF A6

for

toxic metabolite production

Aim of the investigation

Fungi are known to produce many secondary metabolites. Some of these metabolites are considered mycotoxins. The aim of this investigation was to analyse one culture extract of *Aspergillus niger* and two samples of a fermentation batch for toxic metabolites.

Cultivation and extracting of the strain and sample were performed at the Centraalbureau voor Schimmelcultures in Utrecht. The extracts were sent to Prof. Jens C. Frisvad (University of Denmark, Lyngby), who analysed the metabolites and compared them with all important toxins which may be significant.

Methods

The following samples were examined:

***Aspergillus niger* DS47447 (EPO712-9)**
Fermentation broth JLL 03 006 IDF C1
Filtrated UF concentrate JLL 03 066 IDF A6

The strain was cultured on the following media which are given the most optimal expression for the production of secondary metabolites: Czapek yeast autolysate agar (CYA), Blakeslee malt agar (MEA), yeast extract sucrose agar (YES) and oatmeal agar (OA) (Frisvad and Filtenborg, 1989; Frisvad, 1993). All cultures were incubated for 14 days in darkness at 24°C.

For metabolite analysis, the contents of each plate were combined and by the method described in Frisvad and Thrane (1987) and analysed by high performance liquid chromatography (HPLC) with diode array detection (DAD) (Frisvad and Thrane, 1993). The same extraction procedure was followed for the fermentation samples.

The metabolites found were compared to a spectral UV library made from authentic standards run at the same conditions (the maximal similarity was a match of 1000), and retention indices were compared with those of standards.

Results:

The strain *Aspergillus niger* DS47447 (EPO712-9) showed several unknown metabolites and no known mycotoxins were found.

In the samples of the fermentation broth JLL 03 006 IDF C1 and Filtrated UF concentrate JLL 03 066 IDF A6 ergosterol was found and no mycotoxins.

Conclusions

When compared to a spectral UV library made from authentic standards of all important fungal toxins which may be of significance, no mycotoxins were found (Cole and Cox , 1981; Smith and Moss, 1985).

References:

Cole, R.J. & Cox, R.H. (1981). Handbook of toxic fungal metabolites. Academic press, New York.

Frisvad, J.C. & Filtenborg, O. 1989. Terverticillate penicillia: chemotaxonomy and mycotoxin production. Mycologia **81**: 837-861.

Frisvad, J.C. & Thrane, U. 1987. Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). Journal of Chromatography **404**: 195-214.

Frisvad, J.C. & Thrane, U. 1993. Liquid column chromatography of mycotoxins. In: Betina, V. (ed.): Chromatography of mycotoxins. Techniques and applications. Journal of Chromatography Library **54**: 253-372. Elsevier, Amsterdam.

Smith, J.E. & Moss, M.O. (1985). Mycotoxins. Formation, analysis and significance. John Wiley & Sons, Chichester.

Utrecht, 29 September 2003

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Note: The HPLC spectra of *Aspergillus niger* DS47447 (EPO712-9) and the Fermentation broth JLL 03 006 IDF C1, Filtrated UF concentrate JLL 03 066 IDF A6 are filed in the Centraalbureau voor Schimmelcultures archive and can be available for inspection.