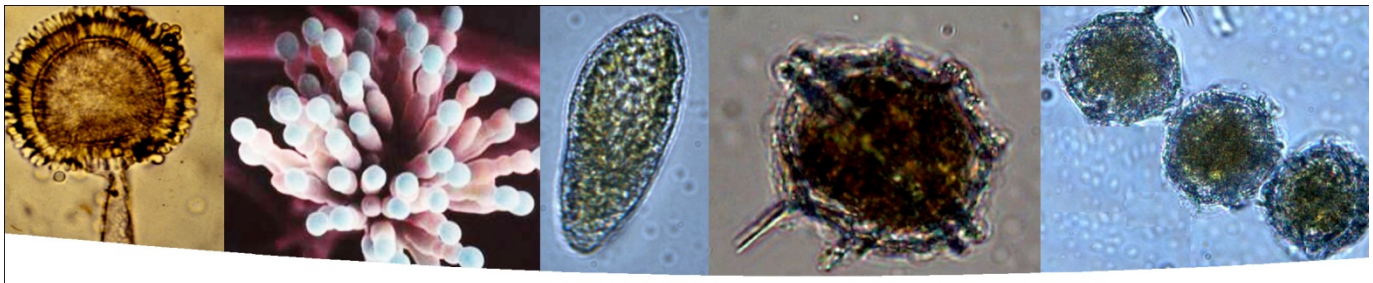

PROCEEDINGS

VI LATIN AMERICAN CONGRESS OF MYCOTOXICOLOGY
and
II INTERNATIONAL SYMPOSIUM
ON ALGAL AND FUNGAL TOXINS FOR INDUSTRY

June 27 to July 1, 2010

Hotel Fiesta Americana
Merida Yucatan

Magda Carvajal Moreno
Chairwoman

SOCIEDAD LATINOAMERICANA
DE MICOTOXICOLOGÍA
FUNDADA EN 1992

UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO
POR MI RAZA HABLARA EL ESPIRITU

Instituto de Biología UNAM

International Agency for Research on Cancer
Centre International de Recherche sur le Cancer

NEOGEN

ILSI International Life Sciences Institute

r-biopharm

TÉCNICA MINERAL

Tecna R&D•Diagnostics - Biotechnology www.tecnalab.com

Agilent Technologies

ROMER Romer Labs®

Poster Section VIII: Mycotoxin Methodology. Groups 15 and 16.

Wednesday June 30

Group 15 (P-76 to P-81): Each presentation in 10 min.

P-76 RAPID HPLC METHOD FOR SIMULTANEOUS DETECTION OF AFLATOXINS AND CYCLOPIAZONIC ACID FROM *ASPERGILLUS* SECTION *FLAVI*

Célia Soares[a], Paula Rodrigues[a,c], Otniel Freitas-Silva[a,b], Luís Abrunhosa[a], **Armando Venâncio[a]**

[a] Institute for Biotechnology and Bioengineering, Centre of Biological Engineering , University of Minho, Campus Gualtar, 4710-057, Braga, Portugal
[b] Embrapa Food Technology. Av das Américas, 29501, 23.020-470, Rio de Janeiro, Brazil
[c] CIMO - Escola Superior Agrária de Bragança, Campus Santa Apolónia, 5301-855 Bragança, Portugal

*Tel:00351+253604400 email:avenan@deb.uminho.pt

Background: Mycotoxins are secondary metabolites produced by moulds and are an important world-wide food safety concern. Among the most relevant mycotoxigenic producer fungi are some *Aspergillus* species in particular those belonging to the *Aspergillus* section *Flavi.* These are known to produce the highly carcinogenic aflatoxins in agricultural commodities. Due to its impact in animal and human health, these species are among the most intensively studied ones, being well known producers of aflatoxins (AFB_1, AFB_2; AFG_1 and AFG_2) and cyclopiazonic acid (CPA). Aflatoxins are mainly produced by some strains of *Aspergillus flavus* and *Aspergillus nomius* and by most, if not all, strains of *Aspergillus parasiticus*. On the other hand, cyclopiazonic acid, which naturally occurs in a large variety of crop products as a co-contaminant with aflatoxins, is mainly produced by *Aspergillus flavus* strains. Together they have been shown to cause health problems in animals and humans, resulting in important economic losses. The production of CPA by *Aspergillus* section *Flavi* may also be routinely used for identification purposes since *A. parasiticus*, *A. flavus* and *A. nomius*, exhibit different mycotoxin profiles. The detection and quantification of both these mycotoxins is usually done separately by HPLC with UV detection for CPA and fluorescence detection after post-column derivatization for aflatoxins. There isn't a chromatographic method available to detect simultaneously CPA and the main four aflatoxins.

Aim: To be able to detect aflatoxins and cyclopiazonic acid in a single HPLC run.

Materials and methods: Twenty two strains belonging to *Aspergillus* section *Flavi* were tested for aflatoxins and CPA production in Czapek Yeast Autolysate agar medium (CYA). Strains were inoculated on 6 cm diameter plates and incubated at 25 °C for 12 days in the dark. Three 8 mm diameter plugs were extracted with methanol and filtered. Extracts were analysed using a HPLC system equipped with a Jasco FP-920 fluorescence detector (372 nm excitation wavelength; 462 nm emission wavelength) and a photochemical post-column derivatization (PHRED unit - Aura Industries, USA). Chromatographic separations were performed with a C18 column (Knauer eurospher 100-5, 4 mm x 250mm, 5 µm) and an amino column (Knauer, 4.6 mm x 250 mm, 5 µm), fitted with a precolumn with the same stationary phase. The mobile phase was pumped at 0.8 mL min^{-1} and consisted of an isocratic

programme as follows: methanol/4mM zinc sulphate (65:35, v/v), pH 5. The injection volume was 50 µL. Samples were taken as positive for each of the toxins when yielding a peak at a retention time similar to each standard, with a height five times higher than the baseline noise. CPA standard was supplied by Sigma (St. Louis, MO, USA). Aflatoxins standard was supplied by Biopure (Austria).

Results and Discussion: Under the tested conditions, the amino column generated a chromatogram where it was only possible to discriminate CPA from the total aflatoxins. On the other hand, the C18 column separated even further, allowing the separation of CPA from AFGs and AFBs. With this column the retention times of AFGs, AFBs and CPA were respectively 10, 11 and 16 minutes. The results obtained with the fungal extracts are consistent with the results previously obtained with the common methodology. Data from these assays will be presented and discussed.

Conclusion: This methodology can be used to detect simultaneously both mycotoxins (aflatoxins and cyclopiazonic acid) in fungi cultures using a single HPLC run, even though the separation of the four aflatoxins is still insufficient.

References: Maragos, C.M., 2009(a). Photolysis of cyclopiazonic acid to fluorescent products, World Mycotoxin Journal. 2: 77-84.

Maragos, C. M. 2009(b). Photoreaction of indole‐containing mycotoxins to fluorescent products. Mycotoxin Reserch. 25, 67‐75.

Rodrigues, P., Venâncio, A., Kozakiewicz, Z., Lima, N. A., 2009. A polyphasic approach to the identification of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* Section *Flavi*. International Journal of Food Microbiology, 129, 2, 187-193.

Acknowledgements: Célia Soares was supported by a grant from Fundação para a Ciência e Tecnologia (reference SFRH / BD / 37264 / 2007).

Luís Abrunhosa was supported by a grant from Fundação para a Ciência e Tecnologia (reference SFRH/BPD/ 43922/2008).

Paula Rodrigues was supported by grants from Fundação para a Ciência e Tecnologia (references SFRH/BD/28332/2006 and SFRH/PROTEC/49555/2009)