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# Study of a room temperature phosphorescence phenomenon to allow the detection of aflatoxigenic strains in culture media

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**Abstract**

A novel screening method based on room temperature phosphorescence (RTP) for the visual detection of aflatoxigenic strains from *Aspergillus* genus is described. Strains were cultured on media widely used in food mycology to which methyl-β-cyclodextrin plus bile salts (0.6% sodium deoxycholate) were added. Aflatoxin production was readily detectable after 3 days of incubation at 28 °C by RTP emission from the mycelium of aflatoxigenic strains observed after exposure to UV light. The method was tested on thirty-two *Aspergillus* sp. strains. The phosphorescence phenomenon was reproduced *in vitro* by immobilizing aflatoxin $B_1$ on ion exchange resin beads.


*Keywords:* Aflatoxin production; *Aspergillus* sp.; Cyclodextrin; Bile salts; UV light; Fluorescence; Room temperature phosphorescence

## 1. Introduction

Mycotoxins are naturally occurring compounds produced by molds. Amongst mycotoxins, aflatoxins are carcinogenic, mutagenic, hepatotoxic and immunosuppressive metabolites produced by strains belonging to *Aspergillus flavus* group. Typically, *Aspergillus* molds invade corn, almonds, walnuts, groundnuts and pistachios. Under favourable conditions of substrate, moisture and temperature, some strains of these molds are able to synthesize aflatoxins. In April 2004, an outbreak of jaundice with a high case-fatality rate was reported in Kenya. The outbreak was caused by aflatoxin poisoning from eating contaminated homegrown maize and resulted in 317 cases and 125 deaths (Azziz-Baumgartner et al., 2005).

Although not all Aspergilli are able to produce aflatoxins (Klich and Pitt, 1988), from time to time, new aflatoxigenic species are reported (Kurtzman et al., 1987; Cary et al., 2005; Frisvad et al., 2005); therefore, the development of simple, inexpensive and rapid analytical methods for identifying aflatoxigenic molds is of actual interest.

The native luminescence of aflatoxins arises from their oxygenated pentaheterocyclic structure. Thus, most analytical and microbiological methods for the detection and quantification of aflatoxins are based on this feature.

There is a number of microbiological methods that can be used for the direct visual detection of aflatoxin-producing *Aspergillus* strains, the aim of these procedures is to increase the production of aflatoxins and elicit a bright blue or blue-green fluorescent area surrounding colonies under UV radiation. Complex agar media containing different additives to increase the production of aflatoxins have been implemented for this purpose (Adye and Mateles, 1964; de Vogel et al., 1965; Hara et al., 1974; Lin and Dianese, 1976; Torrey and Marth, 1976; Davis et al., 1987; Dyer and McCammon, 1994; Atanda et al., 2005).

In 2001, the addition of a methylated derivative of β-cyclodextrin (β-CD) to sabouraud dextrose and yeast extract agar was studied for the screening of aflatoxigenic strains (Fente

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Table 1
Production of aflatoxin and other known metabolites by *Aspergillus* sp. strains

| Species | Strain | AF production on APA/CAM | AF production confirmed by HPLC [a] | Metabolites |
|---|---|---|---|---|
| *A. clavatus* | CECT 2673 | − | − | − |
| *A. flavus* | CECT 2949 | − | − | Blasticidin S |
| *A. flavus* | CECT 2686 | − | − | Aflavarin, aflavinines |
| *A. flavus* | CECT 2685 | − | − | − |
| *A. flavus* | CECT 2687 | + | + | Aflatoxin $B_1$, $B_2$ |
| *A. flavus* group | LHICA 1316 | + | + | Aflatoxin $B_1$, $B_2$ |
| *A. flavus* group | LHICA 876 | + | + | Aflatoxin $B_1$, $B_2$ |
| *A. flavus* group | LHICA 893 | − | − | – |
| *A. fumigatus* | CECT 2071 | − | − | Fumigacin; glyotoxin |
| *A. ochraceus* | CECT 2917 | − | − | L-Malic acid |
| *A. ochraceus* | CECT 2969 | − | − | Penicillic acid |
| *A. oryzae* | CECT 2095 | − | − | − |
| *A. parasiticus* | CECT 2681 | + | + | Aflatoxins $B_1$, $B_2$, $G_1$, $G_2$ |
| *A. parasiticus* | CECT 2682 | + | + | Aflatoxins $B_1$, $B_2$, $G_1$, $G_2$ |
| *A. penicillioides* | CECT 2074 | − | − | − |
| *A. restrictus* | CECT 2075 | − | − | − |
| *A. sclerotiorum* | CECT 2546 | − | − | – |
| *A. terreus* | CECT 2748 | − | − | − |
| *A. terreus* | CECT 2808 | − | − | − |
| *A. versicolor* | CECT 2814 | − | − | Sterigmatocystin |
| *A. wentii* | CECT 2887 | − | − | Gluconic acid; mannitol; glycerol |
| *E. corrugata* | CECT 2830 | − | − | − |
| *E. nidulans* | CECT 2833 | − | − | – |
| *E. herbariorum* | CECT 2922 | − | − | Auroglaucin; flavoglaucin |
| *E. halophilicum* | CECT 2072 | − | − | − |
| *E. tonophilum* | CECT 2076 | − | − | – |
| *A. aculeatus* | CECT 2968 | − | − | − |
| *A. awamori* | CECT 2907 | − | − | − |
| *A. carbonarius* | CECT 2086 | − | − | – |
| *A. hennebergii* | CECT 2801 | − | − | − |
| *A. niger* | CECT 2088 | − | − | − |
| *A. niger* | CECT 2915 | − | − | − |

+: positive to aflatoxin production; −: negative to aflatoxin production.
[a] HPLC determination of YES chloroform extracts for aflatoxin detection (Jaimez et al., 2003a,b).

et al., 2001). Later on, a practical application of these media for the simultaneous enumeration of yeasts and molds from feeds and diagnostics of aflatoxigenic strains was reported (Jaimez et al., 2003a).

Cyclodextrins (CD) are cyclic (α-1,4)-linked oligosaccharides of a-D-*gluco*-pyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. Experimental results obtained in a number of works (Vázquez et al., 1991; Cepeda et al., 1996; Vázquez et al., 1999; Chiavaro et al., 2001) revealed that β-CDs and their methylated derivatives offer excellent protective cavities for enhancing the fluorescent signal of aflatoxins through the formation of inclusion complexes.

In 2003, the addition of a methylated derivative of β-CD plus sodium deoxycholate (NaDC) to yeast extract agar (YES) was found to be suitable for the identification of aflatoxigenic *Aspergillus* strains; this was achieved through the visualization of a beige ring surrounding the colonies. When this ring was examined under UV light, it exhibited blue fluorescence (Jaimez et al., 2003b). Furthermore, it was observed that aflatoxigenic colonies grown in such environment also emitted room temperature phosphorescence (RTP), when examined in the dark, following excitation with a UV light lamp.

Fluorescence detection has been widely employed in microbiological and analytical techniques for the detection of aflatoxin-producing *Aspergillus* strains, whereas no room temperature phosphorescence method has been reported to date. Compared to fluorimetry, RTP offers some important advantages: the use of phosphorescent probes allow to get rid of the high fluorescent backgrounds observed in *in vivo* measurements due to the native autofluorescence from cells. Moreover, phosphorescent indicators offer chemical stability, long triplet lifetimes and RTP excitation spectra in the near infrared, where biological tissues absorb very little (O'Sullivan et al., 2002; Burke et al., 2003). In addition, these phosphorescent probes can be encapsulated inside dendrimers and develop bio-compatible medical imaging agents (Rietveld et al., 2003). Finally, the selectivity and specificity achievable through RTP detection are better, since phosphorescence is a less common luminescent process than fluorescence; this feature allows developing rapid "phosphoroimmunoassays" without the need of any separation and washing steps (Sidki et al., 1986; Mantrova et al., 1994; Liu et al., 2004, 2005).