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Identification of mycotoxins by UHPLC–QTOF MS in airborne fungi and fungi isolated from industrial paper and antique documents from the Archive of Bogotá

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ABSTRACT

Mold deterioration of historical documents in archives and libraries is a frequent and complex phenomenon that may have important economic and cultural consequences. In addition, exposure to toxic fungal metabolites might produce health problems. In this work, samples of broths of fungal species isolated from the documentary material and from indoor environmental samples of the Archive of Bogotá have been analyzed to investigate the presence of mycotoxins. High resolution mass spectrometry made possible to search for a large number of mycotoxins, even without reference standards available at the laboratory. For this purpose, a screening strategy based on ultra-high pressure liquid chromatography coupled to quadrupole-time of flight mass spectrometry (UHPLC–QTOF MS) under MS^E mode was applied. A customized home-made database containing elemental composition for around 600 mycotoxins was compiled. The presence of the (de)protonated molecule measured at its accurate mass was evaluated in the samples. When a peak was detected, collision induced dissociation fragments and characteristic isotopic ions were also evaluated and used for tentative identification, based on structure compatibility and comparison with literature data (if existing). Up to 44 mycotoxins were tentatively identified by UHPLC–QTOF MS. 34 of these tentative compounds were confirmed by subsequent analysis using a targeted LC–MS/MS method, supporting the strong potential of QTOF MS for identification/elucidation purposes. The presence of mycotoxins in these samples might help to reinforce safety measures for researchers and staff who work on reception, restoration and conservation of archival material, not only at the Archive of Bogotá but worldwide.

1. Introduction

Among the many species of filamentous fungi which exist in nature, some of these are deteriorating agents that may cause biodeterioration of paper and parchment in ancient books and documents (Gallo, 1985; Zotti et al., 2008; Zyska, 1997). These molds can be opportunistic fungi or spoilage fungi in documentary material due to their enzymatic activity (Sterflinger, 2012). It has been reported that over 200 fungal species have been isolated from paper items, although only 5–10% of the total fungal species have been identified (Cappitelli et al., 2010). Some species have been identified as mycotoxin-producing strains with allergenic potential that might put at risk researchers' or staff's health who work in the reception, restoration, conservation and archival processes at libraries and archives (Bennett and Klich, 2003; Bräse et al., 2009). Exposure to mycotoxins is mostly by ingestion, but may also occur by dermal and inhalation routes. The diseases caused by exposure to mycotoxins are known as mycotoxicoses (Bennett, and Klich, 2003; Zain, 2011) and, it has been reported that symptoms depend, among other factors, on the type of mycotoxin, the amount and duration of the exposure, and interactions with other toxic insults (Bennett and Klich, 2003).

Since its foundation in 2004, the Archive of Bogotá (ADB) has established a strain collection of indoor airborne fungi and fungi

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isolated from industrial paper and antique documents from all the governmental archive buildings of this city. Among these isolates, some potentially mycotoxigenic species were found by optical microscopy and molecular biology methods. Therefore, it is necessary to investigate whether these species produce mycotoxins and which type in order to guarantee the health of workers and researchers.

Modern powerful analytical techniques allow investigating the mycotoxins produced by different fungi. Full spectrum acquisition techniques offer the possibility for screening a huge number of contaminants in post-targeted approaches, i.e. without the need of pre-selecting the analytes for method development. An additional value of high resolution mass spectrometry (HRMS) is that it provides accurate-mass full-spectra data with reasonable sensitivity. By using a hybrid quadrupole-time of flight mass spectrometry (QTOF MS) analyzer, it is feasible to record accurate-mass product ion spectra working in MS/MS mode, which is one of the most valuable tools for confirmatory analysis nowadays. Thus, Kildgaard et al. (2014) used ultra-high performance liquid chromatography–diode array detection–QTOF MS (UHPLC–DAD–QTOFMS) providing both accurate mass full-scan MS and MS/HRMS data. The MS/HRMS data were then searched against an in-house MS/HRMS library of ~1300 compounds for unambiguous identification. The methodology was demonstrated on compounds from bioactive marine-derived strains of *Aspergillus, Penicillium*, and *Emericellopsis*.

Another possibility is the MS^E approach, which allows collecting simultaneously information on both (de)protonated molecules and their fragment ions, by acquiring data at low and high collision energy in a single injection. Furthermore, practical parameters, such as isotopic patterns and double bound equivalent (DBE), can be used to facilitate the process of identification/confirmation. With all these possibilities, the tentative identification of the compound detected is commonly feasible, even without reference standards (Ibáñez, 2013; Hernández et al., 2011, 2012).

In this work, ultra-high performance liquid chromatography (UHPLC) coupled to QTOF MS has been applied for identification of mycotoxins in fungal culture broths from ADB. Illustrative examples of the compounds detected and tentatively identified in the samples are presented to demonstrate the potential of the approach applied for investigation of large number of mycotoxins in one single analysis. For this purpose, we used a customized database containing the elemental composition of around 600 mycotoxins previously reported in the literature. As no reference standards were available in our laboratory for confirmation of the tentatively identified compounds, in a subsequent step the sample extracts were re-analyzed at the Department for Agrobiotechnology, University of Natural Resources and Life Sciences (Vienna, BOKU), using a targeted UHPLC–MS/MS method for more than 500 target analytes (Malachová, 2014).

2. Materials and methods

2.1. Reagents and chemicals

Glucose, aspartic acid ($C_4H_7NO_4$), KH_2PO_4, $MgSO_4 \cdot 7H_2O$, NaCl, KCl, $FeSO_4 \cdot 7H_2O$ and $ZnSO_4 \cdot 7H_2O$ were purchased from (Panreac Química S.L.U., Barcelona, Spain) and the yeast extract was purchased from MP Biomedicals (Santa Ana, CA, USA). *Potato Dextrose Agar* (PDA) medium, Sabouraud agar medium and Sabouraud broth medium were from MP Biomedicals Santa Ana, CA, USA). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q plus system from Millipore (Bedford, MA, USA). HPLC-grade methanol (MeOH), sodium hydroxide (NaOH) and formic acid (HCOOH) were acquired from Scharlau (Barcelona, Spain). Leucine enkephalin, used as the lock mass, was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Strains and media for analysis mycotoxins

Several potentially mycotoxigenic indoor airborne fungi and fungi isolated from industrial paper and antique documents from the Archive of Bogotá were used in this study: *Penicillium chrysogenum* (isolates 5D and DC01), *Penicillium purpurogenum* (isolate 66A), *Aspergillus tamarii* (isolate 40A), *Aspergillus flavus* (isolates 81D and 79A), *Aspergillus niger* (isolate 39A), *Aspergillus fumigatus* (isolates DC08, DC20, 13A and 74D) and *Aspergillus versicolor* (isolates 14D and 52D), *Aspergillus ochraceus* (isolate 78A) *Stachybotrys chartarum* (isolates 12D and 35A) and *Fusarium equiseti* (isolates 50A, 45A and 7D). The isolates named with D correspond to fungi isolated from industrial paper, those with DC to antique documents from the seventeenth century and those with A to indoor airborne fungi.

Stock cultures were maintained on *Potato Dextrose Agar* (PDA) medium. For the secondary metabolites (mycotoxins) induction experiments 20 mL of modified Czapek-Dox medium at pH 4.5 were used: 10 g/L glucose, 0.54 g/L aspartic acid ($C_4H_7NO_4$), 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7\ H_2O$, 0.25 g/L NaCl, 0.25 g/L KCl, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 0.01 g/L $ZnSO_4 \cdot 7H_2O$, and 1 g/L yeast extract. Fungi were cultured at 28 °C, 140 rpm, in darkness for 15 days. The culture broths were collected in 50 mL tubes (two replicates per fungal isolate) and kept at −20 °C for further analyses at the Research Institute for Pesticides and Water, University Jaume I (Spain).

2.3. Sampling and isolation of filamentous fungi

The filamentous fungi evaluated in the present study belong to the strain collection of the Archive of Bogota, which is composed by several fungi collected from different sources: (i) Industrial paper with an advanced degree of biodeterioration (Rojas et al., 2009), (ii) antique books from the 17th, 18th and 19th century, made of manual paper, parchment and/or leather and (iii) indoor airborne of several public archive buildings of Bogota (Colombia) (Cruz Ramírez et al., 2012). Fungi of the documentary materials were taken carefully with a cotton swab or scalpel and stored in sterile Petri dish until further processing; airborne fungi were collected using a microbial air sampler (Merck MAS100 NT). All fungi were cultured on different media, mainly PDA medium and Sabouraud agar medium, incubated at 28 °C for 1–2 weeks and isolated by the single-spore method (Ho and Ko, 1997).

2.4. Molecular and morphological identification

All the fungal isolates were identified to the genus level by optical microscopy, using lactophenol cotton blue stain (BD, Franklin Lakes, NJ, USA) of reproductive structures (if visible) and then confirmed by molecular identification.

Isolated fungi were cultured in Sabouraud Broth supplemented with glucose 2% per five days at 28 °C and 150 rpm. Each biomass produced was filtered through sterile Whatman® paper and washed twice with sterile distilled water, and kept at −80 °C until processing. Before DNA isolation, each recovered biomass was pulverized with liquid nitrogen. DNA isolation was performed according to Dellaporta et al. (1983), and DNA integrity and purity were verified by agarose-gel electrophoresis and spectrophotometry A260/280, respectively.

The molecular identification of the fungal isolates was performed by ribosomal DNA loci amplification and sequencing. The PCR amplification was carried out in a C1000 thermal cycler (BioRad, Hercules, CA, USA). For further details, see Supplementary Information