MYCOBIOTA AND FUMONISIN CONTAMINATION IN DRIED FRUITS OF DIFFERENT ORIGIN

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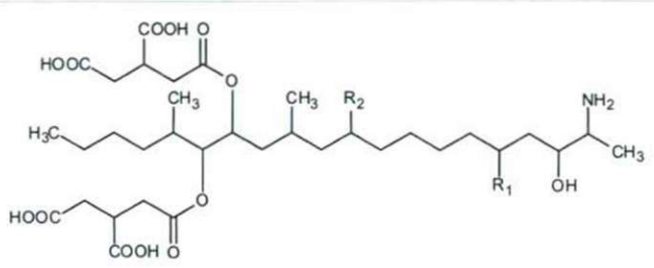
ABSTRACT

Fumonisins are carcinogenic mycotoxins which were originally identified in *Fusarium verticillioides*. According to recent findings, fumonisins are also produced by some black *Aspergillus* species including *Aspergillus niger* and *A. awamori*. Aspergilli are able to produce fumonisins in high quantities on agar media with low water activities. Data on the occurrence and role of this species in fumonisin contamination of agricultural products with high sugar content are needed to clarify the importance of *A. niger* in human health. The mycobiota and fumonisin contamination of various dried fruit samples collected form different countries were examined to clarify the role of black Aspergilli in fumonisin contamination of such products. All except two of the examined raisin samples were contaminated with black Aspergilli. Species assignment of the isolates was carried out using sequence analysis of part of the calmodulin gene. The range of fumonisin isomers present in the raisin samples, and produced by *A. niger* and *A. awamori* isolates collected from dried vine fruits was also examined using reversed-phase high-performance liquid chromatography/electrospray ionization – ion trap mass spectrometry. Among the *A. niger/A. awamori* isolates identified, 67% produced fumonisins. The isolates produced several fumonisin isomers also present in the dried vine fruit samples, including fumonisins B_{1-4}, 3-epi-FB_3, 3-epi-FB_4, iso-FB_1, and two iso-FB_{2-3} forms. Most of these isomers have previously only been identified in *Fusarium* species. The average fumonisin content of the 7 dried vine fruit samples which were found to be contaminated by potential fumonisin producing black Aspergilli was 7.22 mg kg^{-1}. Our data indicate that *A. niger* and *A. awamori* are responsible for fumonisin contamination of dried vine fruits worldwide. The observed levels of contamination are alarming and pose a new threat for food safety. Preliminary data also indicate that fumonisin contamination of other dried fruits including figs and dates, and that of onions are also caused primarily by black *Aspergillus* species. Further work is in progress to examine the role of black Aspergilli in fumonisin and ochratoxin contamination of agricultural products.

1. INTRODUCTION

Fumonisins are carcinogenic mycotoxins which were originally identified in *Fusarium verticillioides* (teleomorph *Gibberella moniliformis*). Fumonisins are mycotoxins produced by several species of the genus *Fusarium*, including *Fusarium proliferatum, F. subglutinans, F. oxysporum* and *F. globosum* (3, 10)(Table 1). Fumonisin B_1 is predominant in most Fusaria, FB_2 and FB_3 usually account for up to 15-25% and 3-8%, respectively, while FB_4 is normally present in insignificant amounts (Fig. 1). Regarding the toxicity of fumonisins, high levels of fumonisin contamination in home-grown maize were found to be associated with high prevalence of human esophageal cancer in several parts of the world including Transkei region in South Africa, LinXian province in China, Northern Italy, Mazandaran and Isfahan provinces in Iran, Southeastern USA, India, Kenya, Zimbabwe and Brazil (12). Fumonisins

have also been shown to be involved in leucoencephalomalacia in horses, pulmonary edema in pigs, and liver cancer and neural tube defects in experimental rodents (12).



	R_1	R_2	Formula
Fumonisin B_1	OH	OH	$C_{34}H_{59}NO_{15}$
Fumonisin B_2	OH	H	$C_{34}H_{59}NO_{14}$
Fumonisin B_3	H	OH	$C_{34}H_{59}NO_{14}$
Fumonisin B_4	H	H	$C_{34}H_{59}NO_{13}$

Figure 1. Chemical structures of the main fumonisin isomers

Table 1. Fungi able to produce fumonisins (7, 10, 14)

Fusarium* species** **Section *Liseola: *F. verticillioides, F. proliferatum, F. fujikuroi, F. sacchari, F. subglutinans (?), F. anthophilum, F. globosum, F. thapsinum* **Section *Dlaminia***: *F. nygamai, F. dlamini, F. napiforme (?), F. pseudonygamai, F. andiyazi* **Section *Elegans***: *F. oxysporum* **Section *Arthrosporiella***: *F. polyphialidicum*
***Aspergillus* species** *A. niger, A. awamori*
***Tolypocladium* species** *T. inflatum, T. cylindrosporum, T. geodes*

Table 2. Fumonisin production by various species on different media (2, 7)

Media	*A. niger/A. awamori*	*F. verticillioides*	*T. inflatum*
Dichloran 18% glycerol agar (DG18)	++	-	++
Czapek yeast autolysate agar + 5% NaCl (CYAS)	+++	-	-
Czapek yeast autolysate agar + 20% sucrose (CY20S)	+++	-	-
Yeast extract sucrose agar (YES)	+++	-	+++
Malt extract agar (MEA)	-	+++	++

Potato carrot agar (PCA)	-	+	+
Potato dextrose agar (PDA)	-	+++	+++
Oatmeal agar (OAT)	-	+++	-
V8-juice agar with antibiotics (V8)	+	++	++

Recent findings indicate that species unrelated to Fusaria are also able to produce fumonisins. In a recent study, Pel et al. (8) have identified a putative gene cluster for fumonisin biosynthesis in the phylogenetically very distantly related fungus *Aspergillus niger*, and fumonisin production has also been proved for several *A. niger* isolates came from culture collections, coffee beans, grapes and raisins (2, 6, 14). Another fumonisin producing species, *Aspergillus awamori* has recently been found to represent a phylogenetic species closely related to *A. niger* based on a multilocus sequence approach and AFLP analysis (Perrone et al., submitted). Besides, recently Mogensen et al. (7) have also observed fumonisin production in *Tolypocladium* species. Most of these reports claim that *A. niger* and *Tolypocladium* species produce only fumonisins B_2 and B_4. While *F. verticillioides* produces fumonisins on agar media based on plant extracts such as barley malt, oat, rice, potatoes, and carrots, *A. niger* is able to produce fumonisins in high quantities on agar media with a low water activity (2). Several agricultural products fit this criterion, including dried vine fruits (including raisins, sultanas, currants), dates and figs.

We examined the mycobiota and fumonisin contamination of various dried fruit (raisin, sultana, fig and date) samples collected from different countries to clarify the role of black Aspergilli in fumonisin contamination of such products. We also examined the range of fumonisin isomers present in the samples, and those produced by fungal isolates collected from dried vine fruits using reversed-phase high-performance liquid chromatography/electrospray ionization – ion trap mass spectrometry (RP-HPLC/ESI–ITMS).

2. MATERIALS AND METHODS

2.1. Dried fruit samples and fungal isolates

Altogether 22 dried vine fruit, fig and date samples were collected from various parts of the world. The samples were surface sterilized using 96% ethanol by immersion for 5 min, and placed on malt extract and dichloran-rose bengal medium (5). The plates were incubated at 25°C for 7 days, and black Aspergilli growing on these plates were purified and identified by classical taxonomic methods (9, 11).

2.2. Genotypic analysis

The fungal cultures used for the molecular studies were cultivated and DNA was extracted as described previously (14). Sequence analyses of the partial calmodulin gene were set up as described previously (4). Phylogenetic analysis of the sequences was performed using MEGA version 4 (13) as described previously (14).

2.3. Extraction and analysis of fumonisins

Fumonisins were extracted from 1 g of the samples with MeOH/H_2O (3/1, v/v). Fumonisins were extracted from the fungal cultures according to Frisvad et al. (2), with minor

modifications (14). The extracts were analysed by a hyphenated technique (RP-HPLC/ESI–ITMS) as described previously (1, 14).

3. RESULTS AND DISCUSSION

3.1. Occurrence of black Aspergilli in dried fruit samples

Black Aspergilli have been identified in 84.6% of the dried vine fruit samples, in 4 of the 5 examined fig samples,and in all 4 date samples (Fig. 2). Species assignment was carried out using sequence analysis of part of the calmodulin gene fragment of the isolates. Potential fumonisin producing *A. niger* or *A. awamori* isolates have been identified in 7 of the raisin samples, in 3 of the fig samples, and in only one of the date samples (14; data not shown).

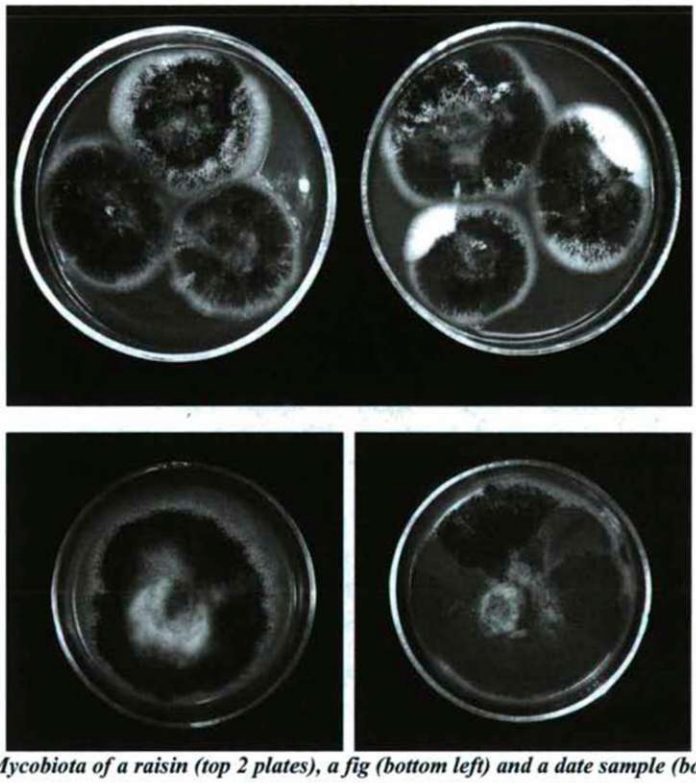


Figure 2. Mycobiota of a raisin (top 2 plates), a fig (bottom left) and a date sample (bottom right)

3.2. Detection of fumonisins in dried fruit samples

RP-HPLC/ESI–ITMS analysis of the dried fruit samples have been carried out to examine the amount and distribution of fumonisin isomers in the samples. Several fumonisin isomers were detected in all the samples, including fumonisins $B_{1\text{-}4}$, 3-epi isomers of FB_3 and FB_4, iso-FB_1, iso-$FB_{2,3}$, FB_5 and iso-FB_5 (14; Fig. 3). The detection of the same isomers in the dried fruit samples and in the fungal cultures indicates that *A. niger* and *A. awamori* are probably responsible for fumonisin contamination of dried fruits including raisins and figs worldwide. The observed levels of contamination are alarming and pose a new threat for food safety.

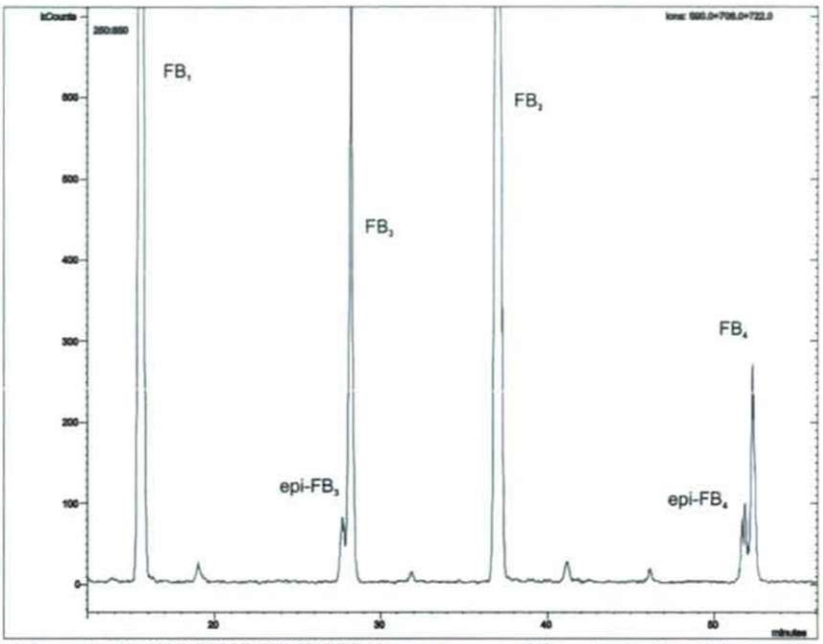


Figure 3. RP-HPLC/ESI–ITMS EIC chromatogram of the extract of a raisin sample

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