Journal of Industrial Microbiology & Biotechnology (1998) 20, 53–54

Mycotoxins of *Alternaria alternata* produced on ceiling tiles

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The production of mycotoxins by *Alternaria alternata* in cellulosic ceiling tiles was examined with thin-layer chromatography and high-performance liquid chromatography procedures. Alternariol and alternariol monomethyl ether were found in ceiling tile extracts, whereas extracts of control rice cultures of all three isolates produced these mycotoxins plus altenuene and altertoxin I. Extensive fungal growth and mycotoxin production occurred in the ceiling tiles at relative humidities of 84–89% and 97%.

Keywords: *Alternaria*; mycotoxin; ceiling tiles

Alternaria alternata is one of the most common saprophytes on a wide variety of field crops where it produces post-harvest decay of fruits, grains, and vegetables worldwide [6]. Its conidia are abundant among the airspora, especially during ripening and harvesting of cereal crops [5] and conidia are commonly found in soil, household dust and the indoor air of buildings [6,13]. Allergens of *A. alternata* have long been considered to cause significant respiratory symptoms in patients in the United States and they have been implicated recently in serious cases of respiratory arrest [14]. Allergens may cause asthmatic hypersensitivity reactions of the immediate type (IgE mediated) [12]. Moreover, *A. alternata* may colonize the nasal sinuses and produce discomforting allergic fungal sinusitis [13]. *Alternaria alternata* produces various secondary metabolites including the dibenzo-α-pyrones mycotoxins: alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), and a derivative of tetramic acid, tenuazonic acid (TA) [9–11]. The toxicology of these mycotoxins is ill-defined, but they have been shown to be mutagenic and fetotoxic in mice [3].

We have found extensive growth of *A. alternata* on the cellulosic facing of gypsum wall board and on ceiling tiles in several buildings whose inhabitants have complained of moldy air [13]. Whether *A. alternata* produces mycotoxins with growth on these materials is unknown. This study examines mycotoxin production of three indoor isolates of *A. alternata* grown on cellulosic ceiling tiles.

Alternaria alternata 930814 was obtained in Atlanta, GA from an indoor air sample collected in a restaurant with a single-stage Andersen air sampler (Graseby Andersen, Atlanta, GA, USA). *A. alternata* 930311 and 930440 were isolated respectively from internal surfaces of an insulated ventilation duct and a cellulosic acoustic ceiling tile from a building. This ceiling tile and ceiling tiles from two additional buildings were shown with microscopic procedures described previously [1] to be colonized by *A. alternata*. All the collection sites had been the subject of complaints of poor air quality by occupants.

Cultures were grown on potato dextrose agar in 9-cm diameter petri dishes at 25°C for 8 days. The plates were flooded with 10 ml sterile saline (0.9%) containing 0.5% Tween 80. The resultant conidia suspensions were aspirated and diluted in sterile saline to 10^6 conidia ml^{-1} (microscopic cell counts).

Unused white, cellulosic acoustic ceiling tiles of the same type colonized in the buildings were purchased retail, and cut into sections about 70 cm^2. Sections were autoclaved at 121°C for 60 min. The sections of sterile tiles were submerged in sterile water for 1–5 min. The conidia suspension (1.0 ml) was spread evenly on the surface of the tiles. The inoculated sections were positioned on stainless steel racks or suspended with stainless steel wires in sterile glass vessels. The materials were incubated in the vessels for 28–90 days in the dark at room temperature (about 22°C) with ambient relative humidities (RHs) controlled with various salt concentrations at 78–81%, 84–89%, 97%, and 100% (tiles submerged partially in water) [7].

After visually detectable fungal growth enveloped the tiles, we macerated the tile sections in a blender for 20 s in 150 ml of methanol. This suspension was filtered through Whatman No. 1 filter paper and 80 ml of 5% aqueous ammonium sulphate was added followed by a second filtration through Whatman No. 1 filter paper. This filtrate was extracted three times with 50–100 ml of methylene chloride. The extracts were combined and evaporated to dryness in a Buchi rotary evaporator under reduced pressure at 55°C. The residue was reconstituted to 3 ml with methanol and drawn through a 0.45-μm washed cellulose acetate filter and analyzed by TLC and HPLC [2,15]. Pure mycotoxins (Sigma, St Louis, MO, USA) were employed as standards. At least triplicate experiments were performed. To establish that the cultures were capable of mycotoxin production, we compared extracts of 3-week cultures grown on long grain enriched rice (Riceland Foods, Stuttgart, AR, USA) (200 g) containing 50% moisture as a positive control and uninoculated ceiling tiles as a negative control.

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Received 28 May 1997; accepted 6 October 1997

Table 1 Production of mycotoxins by *Alternaria alternata*

	AOH[a]		AME		ALT		ATX-I	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
A. alternata 930311	480.1	±24.9	750.1	±25.8	194.8	±4.4	51.4	±5.8
A. alternata 930440	840.2	±17.4	224.7	±8.6	54.4	±2.1	111.1	±18.9
A. alternata 930814	510.0	±15.8	885.2	±8.7	60.3	±4.7	74.9	±14.5

[a]AOH, alternariol; AME, alternariol monomethyl ether; ALT, altenuene; ATX-I, altertoxin-I; $n = 3$.

Table 2 Mycotoxins *Alternaria alternata* in ceiling tiles with varying water content

RHs[a]	Isolates					
	930311		930440		930814	
	AOH[b]	AME	AOH	AME	AOH	AME
100%	44.4[c]	39.2	50.4	89.6	41.2	45.6
97%	158.8	188.8	231.2	377.2	184.4	220.8
84–89%	160.0	114.0	352.4	194.0	165.6	135.2
78–81%	9.2	–	31.2	–	22.4	5.2

[a]100% = Saturated ceiling tile (partially submerged in standing water); lower values relate to estimated RH of ambient air ceiling tiles suspended over various saturated salt solutions at room temperature.
[b]AOH, alternariol; AME, alternariol monomethyl ether.
[c]μg of toxin g^{-1} of dry weight ceiling tile: – = no mycotoxin detected; $n = 3$, s.d. $\leq$ 5%.

Production of extensive surface growth with dense conidia production of *A. alternata* occurred on ceiling tiles and the cellulosic facing of gypsum wall board both *in situ* and on ceiling tiles in the laboratory. The representative indoor isolates produced AOH, AME and to a lesser extent ALT and ATX-1 with growth in rice cultures; TA was not detected (Table 1). Only AOH and AME were found in extracts from the colonized ceiling tiles with best production at RHs from 84–97% (Table 2). Reduced mycotoxin production (and reduction in conidiogenesis) occurred when fungi were grown on saturated tiles and on tiles maintained at the lower relative humidity range.

Mycotoxins from *Alternaria* have been identified from various food products [10], and AOH and AME have been reported to be present in conidia of *A. alternata* [8]. The production of AOH and AME with growth on ceiling tiles has not been noted previously. Mycotoxins from *Stachybotrys chartarum*, an agent of stachybotryotoxicosis of humans, can be extracted from colonized ceiling tiles and gypsum wall board and these mycotoxins have been implicated in the sick building syndrome (SBS) [2,4]. We have no direct evidence that associates the indoor growth and production of mycotoxins by *A. alternata* with SBS. At several sites, however, we found *A. alternata* associated with *S. chartarum*. Areas of cryptic colonization by *S. chartarum* when uncovered from under vapor barriers were overgrown with *A. alternata*. Although the spectrum of mycotoxins produced by *A. alternata* with growth on cellulosic ceiling tiles was not as extensive as that produced on rice medium, the possibility exists that the mycotoxins are involved in fungal antagonisms on indoor cellulosic surfaces.

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