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Species-specific profiles of mycotoxins produced in cultures and associated with conidia of airborne fungi derived from biowaste

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Abstract

The potential to produce mycotoxins and non-volatile secondary metabolites was investigated for approximately 250 freshly isolated fungal strains. Among the eleven most relevant species, viz. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. parasiticus*, *A. versicolor*, *Emericella nidulans*, *Paecilomyces variotii*, *Penicillium brevicompactum*, *P. clavigerum*, *P. crustosum*, and *P. polonicum*, a wide range of metabolites partly of toxicological relevance was identified. Several unknown metabolites were found for the less frequent species, which were primarily investigated for chemotaxonomic delimitation from closely related species. The spectra of metabolites in conidial extracts and culture extracts (containing also mycelium and medium) were compared for a limited number of relevant fungi. Some mycotoxins, such as sterigmatocystin in *Emericella nidulans*, were not present in the conidial extracts, though produced by most strains. Fumigaclavine C, tryptoquivaline, and trypacidin, characteristic for *A. fumigatus*, were found in conidial extracts, but highly toxic compounds such as gliotoxin and fumitremorgens were not present. Finally, compounds such as cyclopenol, cyclopenin, and penitrem A being characteristic for certain penicillia, were found in conidial extracts and are therefore assumed to occur in native bioaerosols.

Key words: mycotoxins – airborne fungi – occupational hygiene – chemotaxonomy – identification

Introduction

Airborne fungal contaminants in occupational environments are gaining importance in view of health hazards to workers. In addition to pathogenicity and the allergenic potential, possible health impacts of bioaerosols in waste-handling facilities are discussed from the toxicological point of view. While the relevance of mycotoxins has been intensely studied in connection with the contamination of food and feed, the possible respiratory uptake of mycotoxins by bioaerosols is not yet sufficiently taken into account. Toxic secondary metabolites are expected to be present in or attached to airborne spores, and may thus occur in airborne dust and bioaerosols. Potential health risks can not be estimated reliably, unless exposure to mycotoxins is determined both qualitatively and quantitatively.

The commonest fungal species found in the air of compost facilities were investigated for their capability to produce non-volatile metabolites and mycotox-

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ins in view of estimating their toxicological relevance in air. Some toxicologically relevant species occurring more seldom were also included, since they may be underrepresented in the microbiological exposure assessment due to the method of sampling or isolation. To avoid any influence of metabolite production due to strain degeneration, only freshly isolated strains were tested for mycotoxin production. Kale and coworkers found that strains lacked the ability to produce metabolites (*sec–*) already after 5–12 transfers from wild type and spore colour mutants (*sec+*) of *A. parasiticus* (Kale et al., 1994). The variants were stable and did not revert to the parental characteristics after more than ten transfers. These authors recommended spores rather than mycelium as inocula for the maintenance of wild-type cultural and biochemical characteristics.

Reports on health effects due to mycotoxins have so far only been published in livestock breading or in connection with food-derived intoxication. The toxicity of certain compounds mostly results from animal experiments or food-derived intoxication of humans. In such cases, the mycotoxins were taken up by ingestion. From the viewpoint of environmental and especially occupational hygiene the possible inhalation of mycotoxins has recently become more relevant than ingestion. The mycotoxins are likely to be present in or attached to both airborne spores (conidia) and particles of organic dust. Thus, the present study investigates which mycotoxins can be associated with the conidia of airborne fungi.

Materials and methods

Selection of relevant fungal species

The assessment of species-specific spore counts in different parts of composting plants provided basic information on the abundance of fungi in situ. A detailed description on the profiles of species and species-specific spore counts in different parts of the facility is given by Fischer (2000). In the present study, only the prevalent species were investigated for the production of secondary metabolites. The prevalent species occurring with highest cfu counts were *A. candidus*, *A. fumigatus*, *A. flavus*/*A. parasiticus*, *A. niger*, *A. versicolor*, *Emericella nidulans* (*A. nidulans*), *Paecilomyces variotii*, *Penicillium brevicompactum*, *P. clavigerum*, *P. crustosum*, *P. polonicum*, *P. fellutanum*, *P. glabrum*, *P. islandicum*, *P. roqueforti*, and *P. verruculosum*. Some rather infrequently occurring species, e. g. *A. giganteus*, *P. chrysogenum*, *P. expansum*, and *P. variabile* were included in the study for comparison of mycotoxin profiles. The strains tested in the present investigation were too numerous to be listed here explicitly by their strain numbers. Detailed information on the origin and mycotoxin production are kept in a database at the Institute of Hygiene and Environmental Medicine Aachen (unpublished).

Cultivation of fungal strains

To detect a wide range of metabolites and toxins, each strain was grown on four different media: yeast extract-sucrose agar (YES), Czapek-yeast autolysate agar (CYA), malt extract agar (MEA), and oatmeal agar (OA). The media were prepared according to Samson et al. (Samson et al., 1995) using formulations by Difco Laboratories, Detroit (USA). The strains were three-point inoculated on each of the four media in 9 cm Petri dishes and incubated for 14 days at 22 °C (room temperature) in the daylight. The strains tested were deposited in the culture collection of the Institute of Hygiene and Environmental Medicine under their IHUA number. The fungal strains isolated in the present investigation were screened for mycotoxin production after a maximum of two transfers. After a first transfer the strain was brought into the collection, with the second the media were inoculated for the experiments. The number of transfers was reduced to minimize physiological alterations with regard to metabolite production.

Identification of species

The fungi were identified using accepted methods of mycological laboratory practice and by using current References and determination keys. Critical isolates were identified using several keys and kept under the name for which most traits matched. The fungi were identified according to current taxonomic concepts (Raper and Fennell, 1965; Domsch et al., 1993; Pitt, 1988; Gams, 1980 (quoted by Domsch et al., 1993), Svendsen and Frisvad, 1994; Samson et al., 1995). If necessary, identification was confirmed by chemotaxonomic investigation of the mycotoxins using high performance liquid chromatography according to Frisvad and Thrane (1987) and Fischer et al. (1999). This was particularly helpful for the identification of terverticillate penicillia, in particular the *P. aurantiogriseum* complex and synnematious *Penicillium* species (*P. clavigerum*). For mycologists with restricted experience in *Penicillium* systematics, a distinction of species such as *P. crustosum*, *P. commune*, *P. solitum*, and *P. verrucosum* or *P. aurantiogriseum*, *P. cyclopium*, *P. polonicum*, and *P. viridicatum* is not reliable, if only morphological (microscopy, colony) traits are used. In the present investigation these species could be reliably identified using chemotaxonomic characters. Additionally, isolates of some species were morphologically compared to reference strains from the CBS culture collection to confirm proper identification. In other cases, identification (e. g. the biverticillate species) was discussed with experts from the CBS (Hoekstra, pers. commun. 1997).

Extracts from pure cultures

From each species on each type of medium three agar disks (including mycelium and conidia) 4 mm in diameter were punched out using a cork borer and transferred to 7 ml glass vials with Teflon liner (Supelco). Three ml of chloroform/methanol (2 : 1) containing 1 % formic acid were added and sonicated for 30 min (Branson model 5210). Extracts were filtered through folded filters (Selecta No. 595½ or Whatman 113V) to remove agar pieces and fungal cell walls and transferred into scintillation vials (Wheaton) afterwards, which could best be used for evaporation in the Vortex-

evaporator. One ml of extraction solvent was then added to the 7 ml glass vial to suspend the remaining agar pieces and fungal material. Extracts were evaporated to dryness under nitrogen (N_2) in a Vortex-evaporator (Buchler) at 40 °C for approximately 40 minutes. Residues were suspended in 0.5 ml acetonitrile and filtered through a membrane filter with 0.2 µm pore size (Spartan 13/30, Schleicher/Schuell) to remove colloidal proteins. Samples were stored at –4 °C until analysis.

Extracts from spore suspensions

For the extraction of metabolites associated with or present in conidia, the strains were cultured for 14 days at 22 °C (room temperature) on agar slants containing the above mentioned four media. Two ml of 0.9 % sodium chloride solution with 0.01 % Tween 80 were added to each slant and vortexed for approximately 1 minute to suspend the conidia. Spore suspensions from the four different media were combined to 8 ml, of which 500 µl were used for microscopic quantification of conidia. The remaining 7.5 ml suspension were filtered through a membrane filter of 0.2 µm pore size. The filter was transferred to a 7 ml glass vial with Teflon liner and extracted in the same way as described above for the culture extracts.

Analysis of mycotoxins

Analysis of mycotoxins was performed by high pressure liquid chromatography with diode array detection (HPLC-DAD) according to Frisvad and Thrane (1987). The liquid chromatograph was a Hewlett Packard 1100 series. The column was a Hewlett Packard LiChro Cart® 125-4 packed with LiChrospher® 100 RP-18e (5 µm) in combination with a guard column LiChrospher® 100 RP-18, 5 µm, 4 × 4 mm. Three hundred µl of fungal extracts and 15 µl phenone standard were transferred into 2 ml brown glass vials (Hewlett-Packard). The phenone standards were obtained by the following manufacturers: Propiophenone (Ega Chemie P5,160-5 or > 99 %, Merck-Schuchardt, Hohenbrunn, Germany); butyrophenone (Ega Chemie 12,433-8 or B-5265, Sigma Chemical Co., St. Louis, USA), acetophenone (A1,070-1, 99 %, Aldrich-Chemie, Steinheim, Germany); hexanophenone (H1, 245-5, Aldrich-Chemie, Steinheim, Germany); valerophenone (V65-9, Aldrich Chemical Company Inc., Milwaukee, USA), octanophenone (29,860-3 or 31,977-5, Aldrich-Chemie, Steinheim, Germany), decanophenone (23,200-9, Aldrich Chemical Company, Milwaukee, USA). HPLC analysis was performed as gradient elution using acetonitrile (solvent A) and water (solvent B) both acidified with 0.02 % trifluoroacetic acid; analysis started at 10 % solvent B, increasing to 50 % solvent B within 30 minutes, again increasing to 90 % B in 10 minutes, remaining for 3 minutes at 90 %, then decreasing within 6 minutes to 10 % B, and finally remaining at 10 % for 1 minute. The flow was held at 2 ml/min resulting in a pressure of approximately 170 bar with 10 % B. The retention index (RI) of each compound was calculated on the basis of the retention time according to Frisvad and Thrane (1987). Secondary metabolites were identified using the RI and the UV-vis spectrum (diode array detection) by comparison to reference values given in the References. For the determination of aflatoxin, fluorescence detection (HPLC-FLD) was initially used: extinction wavelength of 365 nm, emission wavelength of 455 nm. The detection via UV-vis absorption was less sensitive than fluorescence detection for this group of metabolites.

Results

Species-specific profiles of mycotoxins in pure cultures on YES

Aspergillus candidus

One metabolite was found in very high quantities, which remained unidentified. The species was variable in regard to production of aerial mycelium and intensity of sporulation on the isolation medium. The variability in macro-morphology was related to differences in metabolite production of various isolates.

Aspergillus flavus, A. parasiticus (Fig. 2)

The species were only sporadically isolated. All isolates tested produced kojic acid, only three out of six isolates produced aflatoxin and aspergillic acid. Asperfuran and nitropropionic acid were found only once.

Aspergillus fumigatus (Fig. 1)

A. fumigatus is a taxonomically well-defined species and can easily be separated from other species by macro-morphological characteristics. The species is widely known as indicator organism for composting processes, where the highest spore numbers in air were observed. The production of non-volatile secondary metabolites was mostly consistent for different isolates. Eleven metabolites were found in culture extracts, a citrinin-like compound, fumagillin, fumigatin, fumigaclavine A, fumigaclavine C, fumitremorgin C, a fumitremorgin metabolite, trypacidin, tryptoquivaline and verruculogen. Tryptoquivaline and trypacidin were produced by all strains of *A. fumigatus*. The highly toxic gliotoxin could only be found in three out of nine strains tested.

Aspergillus giganteus (Fig. 3)

The species produced patulin, nortryptoquivaline and some of its derivatives. The spectrum of metabolites was identical for all tested isolates.

Emericella nidulans (Fig. 4)

The species produced sterigmatocystin, which is known to be a precursor in the aflatoxin biosynthesis. Sterigmatocystin could also be found in the related *Aspergil-*

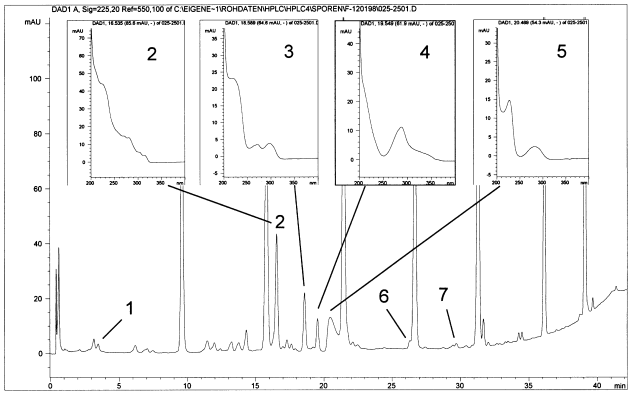

Fig. 1. HPLC-chromatogram of *A. fumigatus* 684.97, with fumigatin [1], fumagillin [7], fumigaclavine C [5], fumitremorgen C [3], tryptoquivaline [2], trypacidin [4], and verruculogen [6].

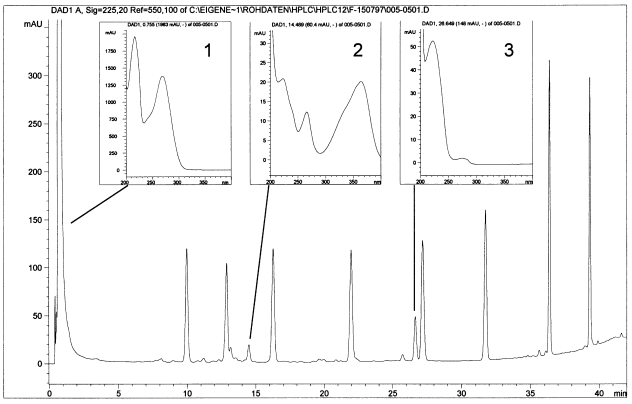

Fig. 2. HPLC-chromatogram of *A. parasiticus* 266.97, with kojic acid [1], aflatoxin B1 [2] and a nitropropionic acid-like compound [3].

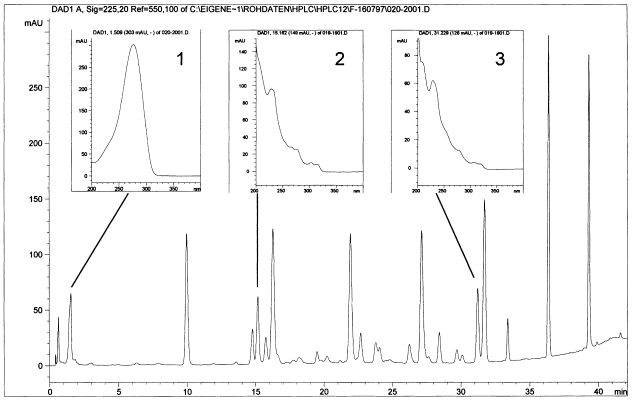

Fig. 3. HPLC-chromatogram of *A. giganteus* 236.97, with patulin [1], a nor-tryptoquivaline derivative [2] and nortryptoquivaline [3].

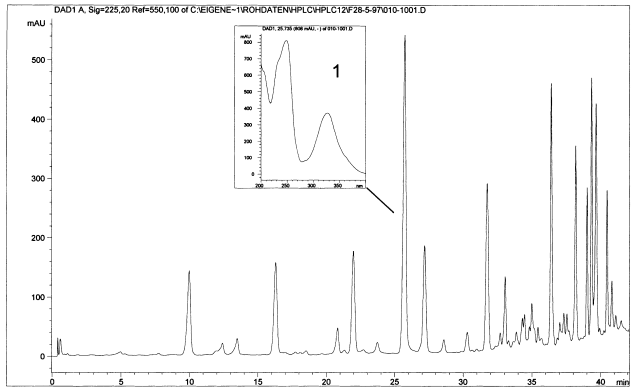

Fig. 4. HPLC-chromatogram of *Emericella nidulans* 9.97. Sterigmatocystin [1] and a number of unidentified metabolites (retention times 30 to 40 min) were characteristic for the species.

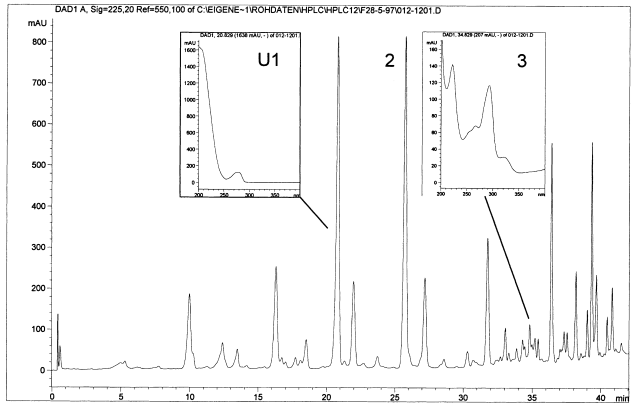

Fig. 5. HPLC-chromatogram of *Aspergillus eburneo cremeus* 145.97, with an unknown metabolite [1], sterigmatocystin [2] and versicolorin A [3].

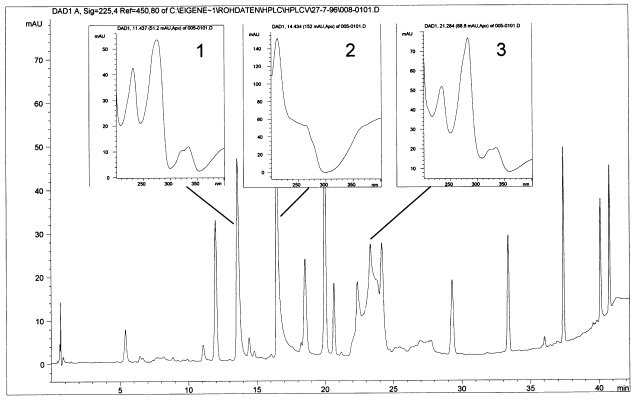

Fig. 6. HPLC-chromatogram of *Aspergillus niger* 424.95, with some tetracycline-like compounds [1, 3] and a naphtho-γ-pyrone [2]. The production of metabolites was inconsistent in different isolates.

lus versicolor, which additionally produced aversin and versicolorin A and B. During the microbial exposure assessment two types of *Emericella nidulans*-group were frequently found. Isolates that formed the teleomorph and showed a bright green conidia colour on DG-18 were classified as *E. nidulans*. Isolates not forming cleistothecia were identified as *A. nidulans*. Furthermore *Aspergillus eburneo cremeus* sensu Raper and Fennell (1965) has been differentiated within the group. The colonies of the latter species had a more grey-green colour due to abundantly produced white to cream-coloured hülle cells.

Aspergillus nidulans

Isolates similar to *E. nidulans* in colony morphology but lacking the teleomorph and showing poor sporulation were classified as *A. nidulans* sensu lato. A further classification of these isolates was not possible. In regard to the production of sterigmatocystin these isolates were not different from *Emericella nidulans*. However, some metabolites with higher retention times (35 to 40 min), which remained unidentified, were found only in strains classified as *A. nidulans*.

Aspergillus eburneo cremeus (Fig. 5)

The species belongs to the *Aspergillus nidulans*-group (section *Nidulantes*). In the isolation medium as well as on the culture media it was different from the latter species in regard to macro-morphology. However, the metabolite production of *A. eburneo cremeus* was similar to isolates classified in *E. nidulans*.

Aspergillus niger (Fig. 6)

The species produced a number of metabolites, most of which could not be identified. Tetracycline-like compounds and naphtho-γ-pyrones were also characteristic for the species. Metabolite synthesis was not consistent within different isolates.

Aspergillus versicolor (Fig. 7)

A. versicolor produced sterigmatocystin as important toxic metabolite. Sterigmatocystin was found in all 12 isolates tested, versicolorin A in 9 and versicolorin B in 11 isolates. Aversin was only found sporadically.

Aspergillus allahabadii B. S. Mehrotra & Agnihotri (Fig. 8)

A. allahabadii also produced sterigmatocystin and additionally some metabolites that were not found in closely related species.

Paecilomyces variotii (Fig. 9)

Patulin and viriditoxin are known for this species (Samson et al., 1995). A series of metabolites was observed to be produced by the isolates tested, but viriditoxin and some viriditoxin-like compounds were found only in isolate 167.95.

Penicillium brevicompactum (Fig. 10)

Most isolates of this species produced asperentin, brevianamide A, mycophenolic acid, and mycophenolic acid-like metabolites. Sporadically, strains were isolated that did only produce meleagrin, and differed in regard to colony morphology and conidial colour. The colonies of this species showed a relatively high variability in regard to colony morphology and conidial colour on the isolation medium (DG-18). Although these differences were less pronounced in pure culture, some strains differed chemotaxonomically.

Penicillium chrysogenum (Fig. 11)

The species produced meleagrin (three isolates) and secalonic acid (2 out of 3 isolates). Roquefortin C and penicillin were reported by Samson et al. (1995), but were not found in the isolates tested here.

Penicillium clavigerum (Fig. 12)

The production of patulin, roquefortin C, isofumigaclavine, and penitrem A is characteristic of the species (Samson et al., 1989; Svendsen and Frisvad, 1994). Patulin and isofumigaclavine-like compounds were found in all 11 isolates tested. The neurotoxic penitrem A was produced by five, roquefortin was found in ten out of 11 isolates. Contrasting with their variable metabolite production, these isolates had a stable colony morphology. The species differs from other closely related synnematous species in that the synnemata are less differentiated and occur besides solitary conidiophores.

Penicillium crustosum (Fig. 13)

The combination of terrestric acid, cyclopenol, cyclopenin, roquefortin C and penitrem A is distinctive. All isolates tested showed the same profile of metabolites, even if their quantities varied. The production of an unidentified metabolite (met. Ω) was also characteristic for *P. crustosum*. Cyclopenol, cyclopenin and penitrem A were produced by all isolates, roquefortine was found in nine, terrestric acid in 13 out of 16 isolates.

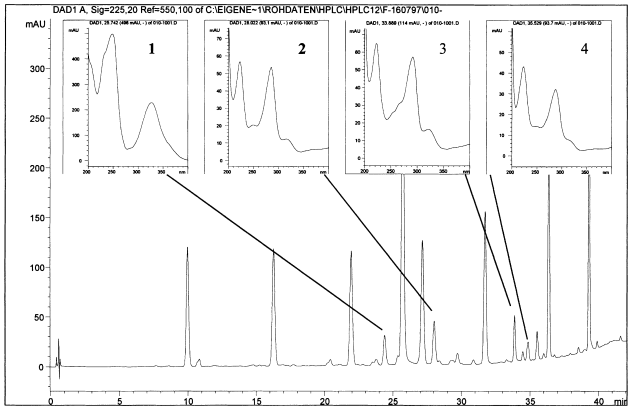

Fig. 7. HPLC-chromatogram of *A. versicolor* 255.97, with sterigmatocystin [1], aversin [2], versicolorin B [3], versicolorin A [4].

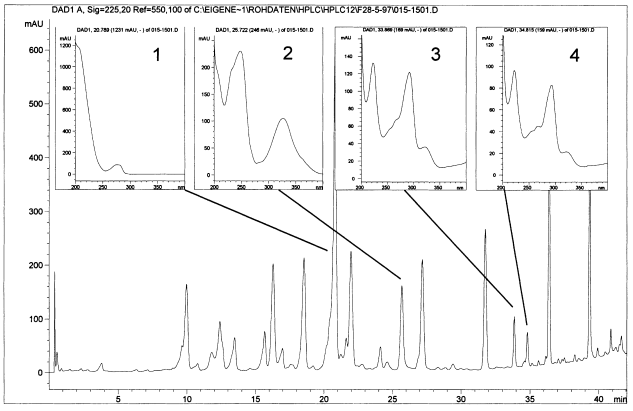

Fig. 8. HPLC-chromatogram of *A. allahabadii* 119.97, with an unknown metabolite [1], sterigmatocystin [2], versicolorin B [3] and A [4].

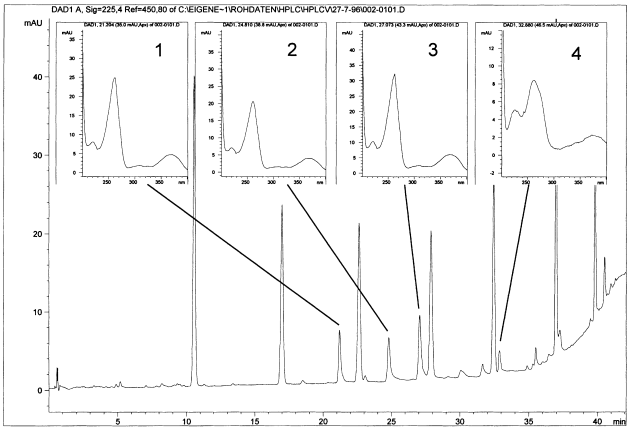

Fig. 9. HPLC-chromatogram of *Paecilomyces variotii* 167.95. The species produced three viriditoxin-like compounds [1–3] and viriditoxin [4] in lower quantities.

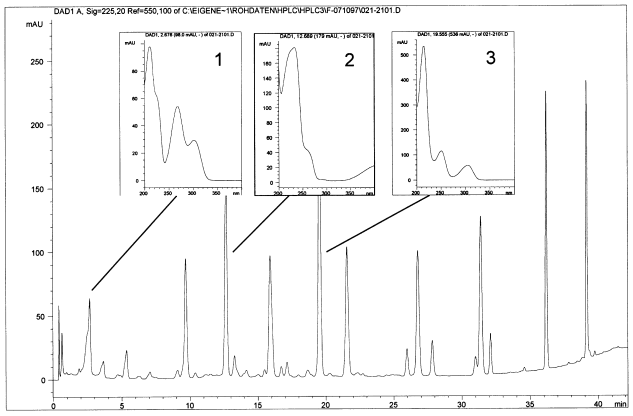

Fig. 10. HPLC-chromatogram of *P. brevicompactum* 595.97, with UV-vis spectra of asperentin [1], brevianamide A [2], and mycophenolic acid [3].

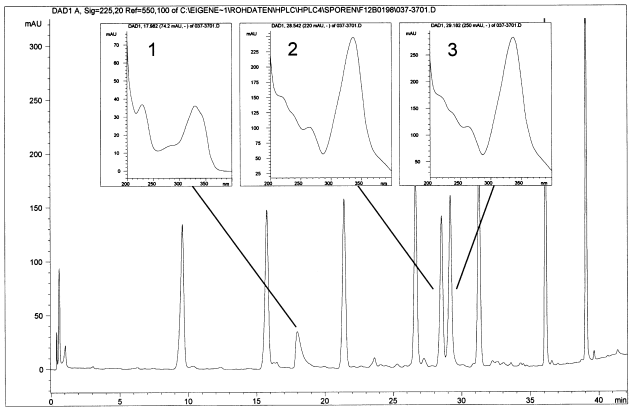

Fig. 11. HPLC-chromatogram of *P. chrysogenum* 619.97 with UV-vis spectra of meleagrin [1], secalonic acid D [3] and a metabolic of it [2].

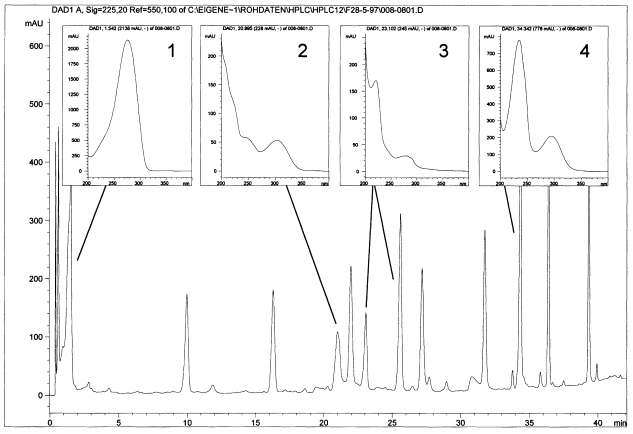

Fig. 12. HPLC-chromatogram of *P. clavigerum* 186.97, with UV-vis spectra of patulin [1], roquefortine C [2], isofumigaclavine-like compounds [3], and penitrem A [4].

Penicillium polonicum (Fig. 14)

Penicillium polonicum is the second most abundant species belonging to the *Penicillium aurantiogriseum*-group. The species is distinct from closely related and morphologically similar species with blue-green conidia by the production of penicillic acid and verrucofortine. Additionally, the species produced cyclopenol, cyclopenin, viridicatin, and verrucosidin. Cyclopenol, cyclopenin, and verrucofortine were found in all 14 isolates tested, viridicatin in 12, penicillic acid and verrucosidin in nine of 14 isolates.

Penicillium expansum

The species produced high quantities of patulin as the most important toxic compound. Roquefortine C was also found in the isolates tested. A number of metabolites, viz. citrinin, communesins, chaetoglobosin C, which were described in References (Samson et al., 1995), could not be identified.

Penicillium fellutanum

For the three isolates tested a significant production of metabolites was not observed. The species was reported to produce citrinin (Laskin and Lechevalier, 1973, cited in Reiß, 1981), which was not found here.

Penicillium glabrum, P. spinulosum

In five (2.97, 109.97, 492.97, 514.97, 604.97) out of eleven isolates trypacidin was detected. Isolates that produced trypacidin contrasted morphologically with the remaining six isolates, that showed extremely weak metabolite production. However, differences in morphology could not be correlated with the chemotaxonomic data. Consequently, isolates of the two species from subgenus *Aspergilloides* could not clearly be identified by means of the chemotaxonomic data. Most metabolites detected could not be identified according to UV-vis spectra and retention indices given in the References. Apart from *A. fumigatus*, only *A. ochraceus* and *Neosartorya fennelliae* were reported to produce trypacidin (Frisvad and Thrane, 1993). Thus, the identification of trypacidin in *Penicillium* subgenus *Aspergilloides* is new.

Penicillium islandicum (Figs. 15, 16)

The four isolates of the species produced a number of metabolites, most of which could not be identified due to the lack of data in References. Three out of four isolates were produced consistently (Fig. 15). All four isolates produced particularly high quantities of rugulosin. *P. islandicum* was reported to produce in addition chrysophanol, islandicin, luteoskyrin, simatoxin, and skyrin, which were not found in the present study. No information was found in References on the molecular structure of simatoxin, which belongs to the group of macrocyclic peptides.

Penicillium purpurogenum (Fig. 17)

The species produced a number of metabolites, most of which could not be identified due to the lack of data in References. Rugulosin was found in all four isolates in varying quantities. The isolates 71.97 and 173.97 generally showed higher metabolic activities. The species was reported to produce kojic acid, ochratoxin and rubratoxin B (Reiß, 1981), none of which was found in the fresh isolates tested here. The identification was confirmed by E. S. Hoekstra (Centraalbureau voor Schimmelcultures, CBS) for some strains (pers. comm., 1997). Rubratoxin B has a similar retention index as sterigmatocystin and must thus elute between 20 and 30 minutes. However, the toxin was not found for the isolates tested.

Penicillium roqueforti (Fig. 18)

The species produced roquefortine C, mycophenolic acid and two metabolites that could not be identified. Roquefortine C was found in two out of three and mycophenolic acid in all isolates.

Penicillium verruculosum

The species was described to produce the tremorgenic verruculogen TR1 (Reiß, 1981), but isolates tested in the present investigation did not show this compound. The three isolates turned out to be a rather weak producer of metabolites.

Comparison of culture and conidial extracts on YES

For the most prevalent species 37 compounds were found in the culture extracts (Table 1). 22 of these compounds were found in the conidial extracts resulting in a percentage of approximately 60 %. Most of these metabolites were consistently present in all isolates tested, except fumagillin, fumigaclavine A, fumigaclavine C, and verruculogen in *A. fumigatus* and asperentin, meleagrin and meleagrin-like metabolite in *P. brevicompactum* (Table 1). In *A. flavus*/*A. parasiticus* only kojic acid occurred in the conidial extracts, in *A. fumigatus* fumitremorgin C, tryptoquivaline and trypacidin were found. Sterigmatocystin and viriditoxin had not been found to be associated with the conidia of *A. nidulans* and *P. variotii*, respectively.

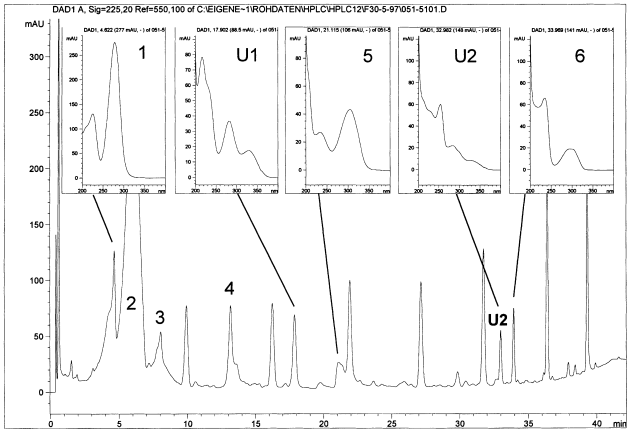

Fig. 13. HPLC-chromatogram of *P. crustosum* 38.97, with UV-vis spectra of terrestric acid [1], metabolite Ω [2], cyclopenol [3], cyclopenin [4], roquefortine C [5], penitrem A [6] and two unknown metabolites.

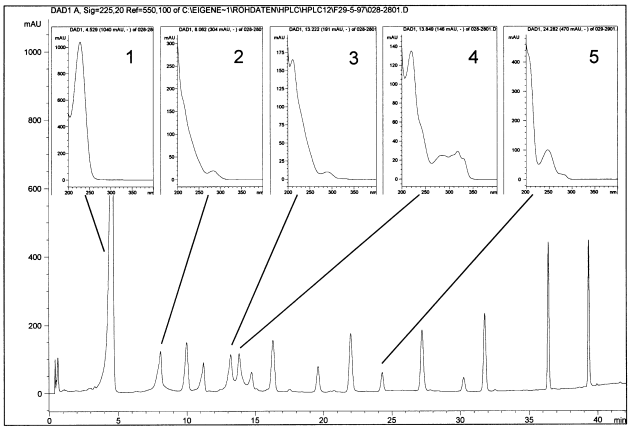

Fig. 14. HPLC-chromatogram of *P. polonicium* 152.97 with UV-vis spectra of penicillic acid [1], cyclopenol [2] cyclopenin [3], viridicatin [4], and verrucofortine [5].

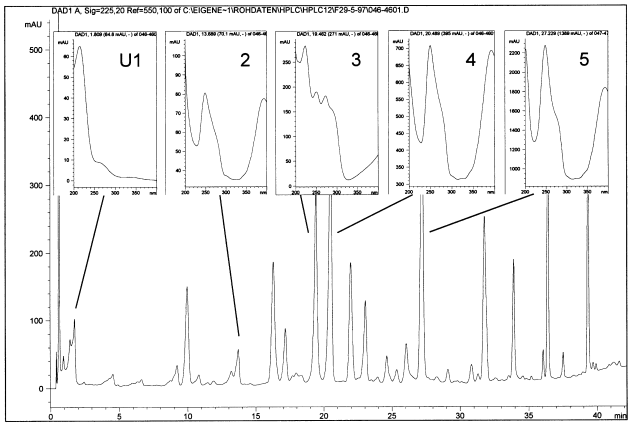

Fig. 15. HPLC-chromatogram of *P. islandicum* 189.97 with UV-vis spectra of an unknown metabolite [U1], a rugulosin-derivative [2], and emodin-like [3], a rugulosin-like compound [4], and rugulosin [5].

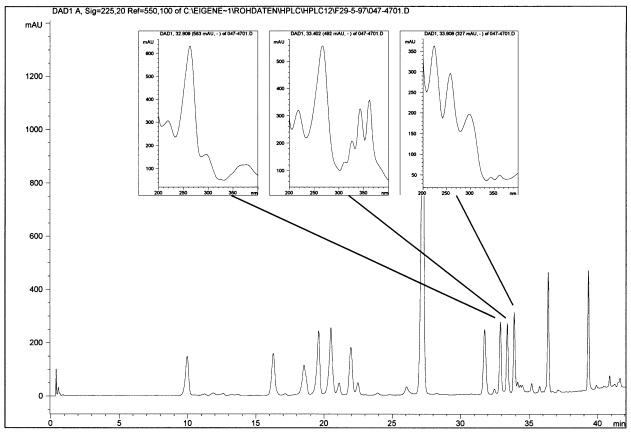

Fig. 16. HPLC-chromatogram of a particular isolate of *P. islandicum* (175.97) producing rugulosin in high amounts and three unknown metabolites.

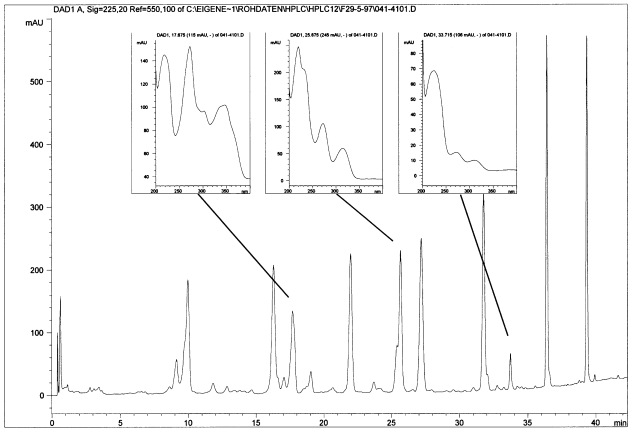

Fig. 17. HPLC-chromatogram of *P. purpurogenum* 71.97 with UV-vis spectra of three unknown metabolites.

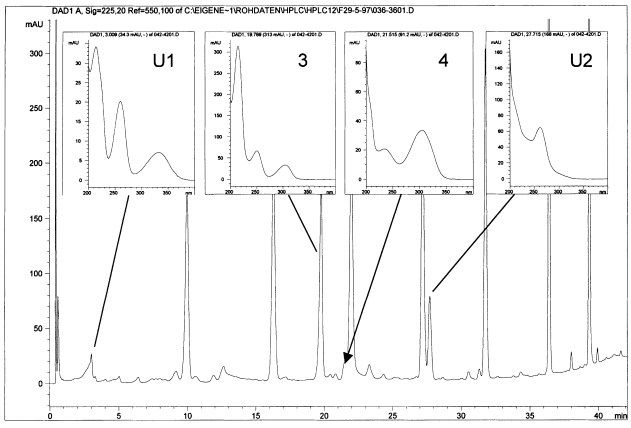

Fig. 18. HPLC-chromatogram of *P. roqueforti* 150.97, with UV-vis spectra of two unknown metabolites [U1, U2], roquefortine C [3], and mycophenolic acid [4].

Table 1. Numbers of strains producing secondary metabolites and mycotoxins on YES medium.

Species	Mycotoxin	Culture extracts:	Conidial extracts:
A. flavus, A. parasiticus	strains tested:	10	3
	Aflatoxin B1	3	0
	Asperfuran	1	0
	Aspergillic acid	3 (A. par.)	0
	Kojic acid	10	3
	Nitropropionic acid	1	0
A. fumigatus	strains tested:	9	9
	Fumagillin	6	2
	Fumigatin	4	0
	Fumigaclavine A	0	1
	Fumigaclavine C	**2**	**8**
	Fumitremorgin C	**8**	**9**
	Gliotoxin 3	0	
	Tryptoquivaline	**9**	**9**
	Trypacidin	**8**	**9**
	Verruculogen	4	1
A. nidulans	strains tested:	20	3
	Sterigmatocystin	17	0
A. versicolor	strains tested:	15	n. t.
	Sterigmatocystin	12	n. t.
	Aversin 3	n. t.	
	Versicolorin A	9	n. t.
	Versicolorin B	11	n. t.
A. niger	strains tested:	9	6
	Naphtho-(-pyrone	3	6
	Tetracycline-like	**9**	**6**
Paecilomyces variotti	strains tested:	5	3
	Viriditoxin	2	0
Penicillium brevicompactum	strains tested:	20	13
	Asperentin	12	8
	Brevianamide	**11**	**11**
	Mycophenolic acid	**14**	**11**
	Meleagrin	4	1
	Meleagrin metabolite	4	1
	Penicillic acid	2	0
	Secalonic acid D	1	0
P. clavigerum	strains tested:	11	1
	Isofumigaclavine-like	**11**	**1**
	Patulin 11	**1**	
	Penitrem A	5	1
P. crustosum	strains tested:	16	3
	Cyclopenol	**16**	**3**
	Cyclopenin	**16**	**3**
	Penitrem A	**16**	**3**
	Roquefortine	9	0
	Terrestric acid	13	0
P. polonicum	strains tested:	14	3
	Cyclopenol	**14**	**3**
	Cyclopenin	**14**	**3**
	Penicillic acid	9	0
	Verrucofortine	**14**	**3**
	Verrucosidine	**9**	**3**
	Viridicatin	12	0

Only the more abundant or most relevant species and their characteristic metabolites are listed. n. t. = not tested.

Brevianamide and mycophenolic acid were present in most spore extracts of *P. brevicompactum* (11 out of 13 strains). In the conidial extracts of *P. clavigerum* an isofumigaclavine-like compound, patulin, and penitrem A were found. Cyclopenol and cyclopenin were found in the conidial extracts of both *P. crustosum* and *P. polonicum*, whereas penitrem A was only present in *P. crustosum* and verrucofortine and verrucosidine occurred in *P. polonicum*.

Discussion

Species-specific potential to produce mycotoxins

The prevalent species, both thermotolerant and mesophilic ones, produced a wide range of secondary metabolites commonly known as mycotoxins. In addition, some species produced metabolites that were not described in the References. *A. candidus* had been reported to produce terphenyllin and xanthoascin (Samson et al., 1995). Both metabolites were not found in any of the isolates tested, but an unknown metabolite (M1) was produced in high quantities. The UV-vis spectrum of M1 could not be found in References and thus information on the toxic impact of this compound is lacking. For *A. niger* a series of tetracycline-like compounds and some others were found, which can only be identified by using additional techniques such as LC-MS and NMR. Although tetracyclines are used as antibiotics they may be of toxicological relevance when occurring in airborne dusts. In *Paecilomyces variotii* viriditoxin-like compounds were not found in the fresh isolates, but occurred in one isolate derived from the culture collection of the IHUA (Fig. 9).

In *Penicillium glabrum, Penicillium fellutanum,* and *Penicillium verruculosum* no characteristic metabolites were found. The biverticillate penicillia such as *P. islandicum* and *P. purpurogenum* produced a series of metabolites, but only rugulosin was identified according to its UV-vis spectrum. It seems that a number of metabolites are still unknown or have not been identified yet. In particular, this concerns higher peptide antibiotics not detected with the present methods but known to be widely distributed among the fungi (Becker, 1996). As it has become obvious that certain biverticillate species can be quite abundant in the air of compost plants, they may become relevant in regard to mycotoxin production. Species such as *Tritirachium oryzae* and *Doratomyces microsporus*, for which higher spore counts were sporadically found *in situ*, were not screened for the biosynthesis of non-volatile metabolites, since toxic metabolites have not been described in References.

Comparison of culture and conidial extracts on YES medium

Species such as *A. fumigatus*, *P. polonicum*, and *P. crustosum* produced certain secondary metabolites consistently when different strains of one species were examined. In *A. niger* and *Paecilomyces variotii* the production was rather inconsistent. In case of *A. niger*, the variability in metabolite production corresponded with varying macro-morphological characteristics of different strains (long or short conidiophores). For the latter two taxa, only few metabolites were identified. Identification of metabolites was difficult because information on UV-vis spectra and retention times available in References was limited. A number of metabolites obtained from culture extracts were also found in extracts of conidial suspensions, thought certain compounds were lacking. Within the species tested a lot of mycotoxins present in culture extracts could also be detected in the conidial extracts.

Those with toxicological relevance such as tryptoquivaline, fumitremorgens, fumigaclavine, tetracyclines, patulin and penitrem A were equally found in the conidial extracts. Some acids such as secalonic acid in *P. chrysogenum* and terrestric acid in *P. crustosum* were not present in the conidial extracts. The production of sterigmatocystin in *E. nidulans* was inconsistent and the compound did not seem to be present in the conidia. Thus sterigmatocystin suspected to be relevant because of high spore counts *in situ* is not likely to be present in bioaerosols. The compound was also produced by *A. versicolor* in culture, but conidial extracts were not tested since sterigmatocystin had also not been found in *Emericella nidulans*. However, these findings show that a series of metabolites and mycotoxins are not only excreted by the fungi into the substrate, but can be expected to be attached to or present in the conidia.

Value of chemotaxonomy for identification

The analysis of profiles of secondary metabolites and mycotoxins can lead to a more precise and reliable determination of the fungi. Several studies on chemotaxonomy have been conducted by Frisvad and co-workers, who discussed whether this technique is suited for the so-called every-day use in laboratories dealing with routine identification (Larsen, 1996). During a study by Fischer approximately 1700 fungal isolates were determined by morphological characteristics and the use of chemotaxonomy turned out to be a useful tool for identification (Fischer, 2000).

For most species tested the respective compounds described in the References were detected. In some penicillia proceeding chemotaxonomic work was most valuable for a precise determination of taxa. *Penicillium polonicum* is thus distinguished from closely related species within the *P. aurantiogriseum*-group, such as *P. aurantiogriseum*, *P. cyclopium*, and *P. viridicatum* (Lund and Frisvad, 1994). A determination on the basis of colony morphology using MEA, CzA, YES, CYA and Creatine-agar according to Samson et al. (1995) often resulted in equivocal results. Especially, the classification according to growth and acid production on Creatine agar was sometimes misleading. *Penicillium clavigerum* was easily distinguished from other synnematous species such as *P. coprobium* by its characteristic profile of mycotoxins initially described by Svendsen and Frisvad (1994). When unexperienced investigators have to determine closely related synnematous species, misidentifications may easily occur, especially if conditions of incubation such as light alter between different laboratories. Thus, the analysis of mycotoxins leads to a more precise identification of such taxa. For most of the fresh isolates the production of mycotoxins was consistent within a species. Strains varying slightly in colony morphology or conidial colour also differed in metabolite production. Thus, the present findings are in agreement with other investigations (Kale et al., 1994; Kale and Bennett, 1990, 1992), which reported differences in morphology to be reflected in altering metabolite production.

Possible toxigenic potential of airborne spores

The experiments carried out show clearly that toxicologically relevant metabolites are likely to be attached to or even present in conidia in the natural environment. If spore counts of the respective species are high in occupational environments, the bioaerosols may contain trace amounts of toxins such as tryptoquivaline, trypacidine, fumigaclavine, and penitrem A. Moreover, many compounds may occur for which a toxic effect has not been described yet, but which are closely related to mycotoxins by their chemical structure. These compounds comprise cyclopenol, cyclopenin, and meleagrin. Since only a few species were tested in the present experiments, further taxa will have to be evaluated for their toxigenic impact in connection with bioaerosols in other facilities and under different circumstances. Among the species relevant here, *A. versicolor*, *P. brevicompactum*, and *P. roqueforti*, and some biverticillate species should be tested in further experiments.

Occurrence and activity of mycotoxins in dusts and bioaerosols

The occurrence of two mycotoxins from *A. fumigatus*, tryptoquivaline and trypacidin, in native bioaerosols has been demonstrated. The detection of these com-

pounds coincided with an extraordinary density of conidia in the air (10^7 cfu/m^3 air) and the two compounds were estimated to occur in the range of ng/m^3 air (Fischer et al., 1999; Fischer, 2000). The fact that other compounds characteristic for certain species were not detected, can possibly be explained in two ways: 1. The concentrations of compounds were too low to be detected with HPLC-DAD. At sampling sites where *A. fumigatus* spore counts ranged one order of magnitude lower, metabolites could not be detected. Spore counts of other dominating species, such as *Paecilomyces variotii* or *Penicillium clavigerum*, ranged approximately two orders of magnitude lower. The respective mycotoxins probably do range below the detection limit. 2. The relevant compounds were not produced under natural conditions. However, recent data on the potential of distinct species to produce mycotoxins on semi-natural substrates indicated, that compounds additional to those already found in native bioaerosols may occur (Fischer, 2000). For instance, cyclopenol, cyclopenin, and penitrem A can be expected in the bioaerosols due to airborne spores of penicillia, whilst sterigmatocystin (*E. nidulans*, *A. versicolor*) is unlikely to occur because it was not present in conidial extracts on YES.

It has become obvious that mycotoxins of certain airborne fungi may be present in the conidia. However, the production of secondary metabolites depends on substrate composition. Therefore it will be necessary to test relevant species for the production of mycotoxins on semi-natural or natural substrates. From the present experiments it can be concluded that a series of mycotoxins are likely to be present in native airborne dust. In further research native bioaerosols from different facilities should be analysed in view of the relevant mycotoxins. The metabolites observed in the bioaerosols may be taken as indicators for the abundance of *A. fumigatus* conidia in the airborne dust, but these metabolites are not the most toxic among those expected to occur.

Until now, the only mycotoxins detected in airborne dusts were trichothecenes of *Stachybotrys chartarum* (Sorenson et al., 1987), aflatoxins of *Aspergillus flavus* (Burg and Shotwell, 1984, Sorenson et al., 1981, 1984), and tremorgens of *A. fumigatus*, but toxins of other species have not been described to occur *in situ*. Some authors suggested that mycotoxins may be involved in the etiology of invasive fungal diseases. For instance gliotoxin, a potent immunosuppressor, has been implicated in the etiology of *A. fumigatus*-induced aspergillosis (Mullbacher and Eichner, 1984). Gliotoxin was also found in *A. fumigatus* culture extracts in the present investigation, but could not be detected in native bioaerosols.

Finally, the detection and quantification of mycotoxins in native bioaerosols is important to estimate health hazards for workers in waste-handling facilities. Further research using more efficient methods for extraction and more sensitive detection techniques will have to further elucidate the levels of mycotoxins in airborne dusts.

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