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Co-occurrence of toxic bacterial and fungal secondary metabolites in moisture-damaged indoor environments

Abstract Toxic microbial secondary metabolites have been proposed to be related to adverse health effects observed in moisture-damaged buildings. Initial steps in assessing the actual risk include the characterization of the exposure. In our study, we applied a multi-analyte tandem mass spectrometry-based methodology on sample materials of severely moisture-damaged homes, aiming to qualitatively and quantitatively describe the variety of microbial metabolites occurring in building materials and different dust sample types. From 69 indoor samples, all were positive for at least one of the 186 analytes targeted and as many as 33 different microbial metabolites were found. For the first time, the presence of toxic bacterial metabolites and their co-occurrence with mycotoxins were shown for indoor samples. The bacterial compounds monactin, nonactin, staurosporin and valinomycin were exclusively detected in building materials from moist structures, while chloramphenicol was particularly prevalent in house dusts, including settled airborne dust. These bacterial metabolites are highly bioactive compounds produced by *Streptomyces* spp., a group of microbes that is considered a moisture damage indicator in indoor environments. We show that toxic bacterial metabolites need to be considered as being part of very complex and diverse microbial exposures in 'moldy' buildings.

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Practical Implications

Bacterial toxins co-occur with mycotoxins in moisture-damaged indoor environments. These compounds are measurable also in settled airborne dust, indicating that inhalation exposure takes place. In attempts to characterize exposures to microbial metabolites not only mycotoxins but also bacterial metabolites have to be targeted by the analytical methods applied. We recommend including analysis of samples of outdoor air in the course of future indoor assessments, in an effort to better understand the outdoor contribution to the indoor presence of microbial toxins. There is a need for a sound risk assessment concerning the exposure to indoor microbial toxins at concentrations detectable in moisture-damaged indoor environments.

Introduction

Indoor environmental conditions characterized by dampness, moisture damage and mold are recognized risk factors for a number of short-term and long-term health effects (Institute of Medicine, 2004; WHO Regional Office for Europe, 2009). Irritation symptoms are most commonly reported, in particular upper respiratory symptoms such as cough, rhinitis, hoarseness and wheezing. Dampness is also a risk factor for exacerbation and new onset of asthma (Institute of Medicine, 2004; Pekkanen et al., 2007). Recurrent respiratory infections, less specific, neurological or general symptoms and rare health outcomes, such as allergic alveolitis, sarcoidosis and rheumatic diseases, have been linked with moisture damage in indoor

environments (Dales et al., 1991; Myllykangas-Luosujärvi et al., 2002; Park et al., 2006).

The nature of causative agents and mechanisms underlying the adverse health outcomes observed in occupants of moisture-damaged buildings remain yet obscure. The research is complicated by the fact that along with chemical exposures, a whole variety of microbial compounds originating from different indoor molds and bacteria may be involved in generating adverse health effects, such as allergenic proteins, structural elements with inflammatory potential and volatile organic compounds (MVOCs). There is a good body of documentation which suggests that nonvolatile microbial toxins – produced as secondary metabolites during microbial growth – may add another critical constituent to the multiple exposures in damp buildings. Fungal and bacterial strains that produce toxic secondary metabolites are present in moisture-damaged indoor environments (Andersson et al., 1998; Engelhart et al., 2002; Fogle et al., 2007). The production of mycotoxins on building materials is well documented (Nielsen, 2003). Microbial spores and cell fragments get airborne and have been shown to contain microbial toxins (Brasel et al., 2005a); subsequently, occupants of contaminated buildings may be exposed through inhalation of indoor air (Brasel et al., 2005b; Gottschalk et al., 2008; Polizzi et al., 2009). Synergistic interactions in modulation of cellular responses upon simultaneous exposure to bacterial and fungal spores, their metabolites and structural compounds have been shown (Huttunen et al., 2004; Islam et al., 2007; Penttinen et al., 2005; Zhou et al., 1999).

Current data on the natural occurrence of toxic microbial metabolites in indoor environments are limited. Most of the analytical methods applied so far were developed to specifically assess the presence of a restricted set of mycotoxins of primary toxicological interest (Bloom et al., 2007; Engelhart et al., 2002; Gottschalk et al., 2008). Recent work from Polizzi et al. (2009) indicated the presence of a multitude of mycotoxins in indoor sample materials. Toxic bacterial metabolites, referred to here as bacterial toxins, have so far been insufficiently addressed in analytical indoor assessments, even though toxigenic bacterial strains are known to be present in indoor environments (Andersson et al., 1998; Mikkola et al., 2007).

In this exploratory study, we analysed building material and dust samples from moisture-damaged indoor environments with a multi-analyte liquid chromatography/mass spectrometry (LC-MS)-based method recently published by Vishwanath et al. (2009), allowing a screening for 159 fungal and 27 bacterial metabolites. We report here the simultaneous detection of multiple microbial toxins from indoor sample materials, including – for the first time in naturally infested sample materials – also bacterial toxins.

Methods

Building material and dust samples

Samples of building materials and various dust sample types were primarily derived from single family homes with severe moisture damage that were investigated in the context of the HoTeS study ('Mold-exposure and health survey'). This is an ongoing study that aims to produce novel information on the occurrence of microbes and microbial metabolites in moisture-damaged indoor environments and their association with health effects in exposed residents. Being conducted in an intervention design, this study investigates the effect of building renovations on exposure and health outcomes. Through collaboration with the Finnish Society for Pulmonary Disabled (Heli), HoTeS recruits families across Finland living in homes affected by indoor conditions of severe moisture damage and dampness that require major repair actions. Self-reported health complaints of the residents linked to spending time in the damaged home are an additional inclusion criterion for the study. Initial building inspections by trained civil engineers are conducted to establish the damage status of the building. Subsequently, renovations are planned and carried out. The families and homes are followed up in the course of this intervention, including both health and microbial exposure measurements before and after the renovations. The herein described sample material includes building materials and different dust sample types from the first nine homes that were recruited to the HoTeS study, collected before renovations. Floor dust was typically sampled in the living room, using a regular vacuum cleaner device and nylon dust sampling socks, as described by Hyvärinen et al. (2006). Settled airborne dust samples were collected in the same way, but from surfaces above floor level, such as bookshelves and similar. The dust bags from the vacuum cleaners used by the families in their homes were additionally collected for analyses. Vacuumed floor dust and dust bag dust samples were size homogenized by sieving through a sterile strainer (pore size approx. 1 mm) to remove the coarse fraction. Vacuumed floor dust and settled airborne dust samples were dried in an exsiccator prior to aliquoting and stored at −20°C. In addition to the sample materials of the HoTeS study, five material samples derived from moisture-damaged public buildings in Sweden – day care centres/kindergartens, a cinema and a university building – were included.

Analyses of microbial metabolites

Building materials and dust samples were analysed with liquid chromatography/tandem mass spectrometry (LC-MS/MS) using the methodology recently published by Vishwanath et al. (2009). The basic list

of target metabolites in this multi-analyte method includes 159 fungal and 27 bacterial metabolites. In brief, the sample materials were extracted in acetonitrile/water/acetic acid (79:20:1, *v*/*v*/*v*), raw extracts were diluted and subsequently analysed without further clean-up. Detection and quantification was done with a QTrap 4000 LC-MS/MS (Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray electrospray ionization source and a 1100 Series HPLC System (Agilent, Waldbron, Germany). Sample preparation, instrumental parameters, method performance characteristics (coefficients of variation, limits of detection, recoveries) and standards used in this multi-target method have been described in detail by Vishwanath et al. (2009). A subset of building material samples was included in an interlaboratory method comparison and was additionally analysed using another LC-MS/MS-based method previously described by Bloom et al. (2007, 2009) targeting selected *Aspergillus* spp. and *Stachybotrys* spp. toxins.

Cultivation and microbial identification

Cultivation and microbial identification was performed on 37 building material samples derived from the HoTeS study. Samples were processed as described previously by Hyvärinen et al. (2002) and plated on 2% malt extract agar and dichlorane glycerol agar (DG18) for fungi, and on tryptone yeast extract glucose agar and Gauze agar for bacteria. Morphological identification and enumeration of fungi and of 'dry' actinobacteria-type colonies were done microscopically.

Results and discussion

Table 1 summarizes the results of the analyses of various indoor sample materials derived from moisture-damaged buildings with a multi-analyte HPLC-MS/MS-based method. In total, 33 different microbial metabolites were detected, with all of the 69 samples analysed being positive for at least one of the targeted analytes. Between 3 and 13 different microbial metabolites were present in the investigated residential homes. Up to 16 metabolites were found in single gypsum board samples of public buildings. Along with well-known indoor mycotoxins, such as sterigmatocystin and satratoxins, we detected in our study metabolites that are novel in the context of naturally infested indoor sample materials. This list includes several fungal metabolites, such as mycophenolic acid or physcion, as well as the bacterial metabolites chloramphenicol, monactin, nonactin, staurosporin and valinomycin (Table 1).

The fungal compounds emodin, enniatin B, beauvericin and the bacterial toxin chloramphenicol were

Table 1 Summary of the microbial metabolite findings in the sample materials collected from moisture-damaged indoor environments

Sampling location	Type and number of sample materials (N)	Microbial secondary metabolites present in the sample materials	
		Fungal	Bacterial
Home 1	BM (9)	Chaetoglobosin A, Emodin, Meleagrin, Roquefortin C, Stachybotrylactam, Sterigmatocystin	Monactin, Valinomycin
Home 2	BM (4), DBD (1), FD (2), SD (1)	Alternariol, Alternariolmethylether, Apicidn, Beauvericin, Emodin, Enniatin A, Enniatin B, Equisetin, Festuclavin, Mycophenolic acid	Chloramphenicol
Home 3	BM (5), DBD (1), FD (2), SD (1)	Beauvericin, Emodin, Enniatin B, Equisetin, Mycophenolic acid, Ochratoxin A, Physcion, Viridicatin	Chloramphenicol, Valinomycin
Home 4	FD (1), SD (1)	Emodin, Enniatin B	Chloramphenicol
Home 5	DBD (1), FD (1), SD (1)	Beauvericin, Emodin, Enniatin B, Equisetin, Mycophenolic acid, Sterigmatocystin	Chloramphenicol
Home 6	BM (5), DBD (1), FD (2), SD (1)	Beauvericin, Emodin, Enniatin B, Mycophenolic acid, Ochratoxin A, Sterigmatocystin	Chloramphenicol
Home 7	DBD (1), FD (1), SD (1)	Beauvericin, Emodin, Enniatin B	Chloramphenicol
Home 8	DBD (1), FD (2), SD (1)	Alternariol, Alternariolmethylether, Beauvericin, Emodin, Equisetin, Enniatin A1, Enniatin B, Physcion	n.d.
Home 9	BM (14), DBD (1), FD (2)	Altenuene, Alternariol, Alternariolmethylether, Beauvericin, Chaetoglobosin A, Emodin, Enniatin A1, Enniatin B, Equisetin, (Hydrolysed Fumonisin B1[b]), Kojic acid, Macrosporin, Physcion, Sterigmatocystin	n.d.
Public buildings 1[a]	BM (2)	Beauvericin, Brefeldin A, Emodin, Enniatin B, Meleagrin, Physcion, Satratoxins G+H, Stachybotrylactam, Sterigmatocystin	Monactin, Nonactin, Valinomycin, Staurosporin
Public buildings 2[a]	BM (3)	Alamethicin, Beauvericin, Brefeldin A, Chaetoglobosin, Chanoclavin, Emodin, Enniatin B, Fumigaclacvine, Meleagrin, Physcion, Roquefortin C, Satratoxins G+H, Stachybotrylactam, Sterigmatocystin, Viridicatin	Monactin, Nonactin, Valinomycin
Total	BM (42), DBD (7), FD (13), SD (7)	28 different fungal metabolites	5 different bacterial metabolites

n.d., not detected; BM, building materials; DBD, vacuum cleaner dust bag dust; FD, vacuumed floor dust; SD, settled airborne dust.

[a]Public buildings 1 include a cinema and a university building; Public buildings 2 include day care centres and kindergartens.

[b]A signal for hydrolysed fumonisin B1 was detected on both MRM (multiple reaction monitoring) transitions, therefore formally complying the criteria for positive identification according to Commission Decision 2002/657; however, because no fumonisin B1 was detected, this finding is highly improbable and can therefore be regarded as interference.

overall most prevalent and were detected in 62%, 57%, 38% and 38% of all samples, respectively (Table 2). The biggest variety of fungal and bacterial metabolites was found in wood-based and gypsum-based building materials, both of which are known to provide a good substrate for fungal and bacterial growth (Hyvärinen et al., 2002). Among house dust samples, floor dust and dust bag dust seemed to equally well capture the variety of microbial metabolites present in the study homes (detection of 12 and 11 different target compounds, respectively; Table 2). Floor dust samples are easily collected in a standardized way by field workers or residents themselves and may therefore be a good sample material for an initial assessment of the indoor presence of microbial metabolites. The amount of toxin(s) detected in a defined floor area may also be useful in calculating a worst case exposure scenario, considering for example an infant re-suspending and inhaling as well as ingesting floor dust while playing on a carpet floor. Naturally, such approach is far from attempting an accurate inhalation exposure assessment.

Six different fungal – emodin, enniatins A1 and B, beauvericin, equisetin, physcion – and the bacterial metabolite chloramphenicol were detected in settled airborne dust (SD) samples. These metabolites are an addition to the list of previously reported, potentially toxic microbial compounds that may occur in indoor

Table 2 Fungal and bacterial metabolites detected in building materials and dust samples collected in moisture-damaged buildings

	Prevalence (%) of microbial metabolites in building materials					Prevalence (%) of microbial metabolites in dust			Overall prevalence (%) $N = 69$
	Wood based $N = 14$	Mineral fibres[a] $N = 10$	Paper based $N = 6$	Gypsum based $N = 5$	Other $N = 7$	Dust bag dust $N = 7$	Floor dust $N = 13$	Settled airborne dust $N = 7$	
Fungal metabolites									
Emodin	71.4	20.0	33.3	60.0	71.4	100.0	92.3	28.6	62.3
Enniatin B	28.6		16.7	80.0	42.9	100.0	100.0	100.0	56.5
Beauvericin	7.1	10.0	16.7	80.0	14.3	71.4	61.5	71.4	37.7
Equisetin	21.4	70.0	16.7		42.9	57.1	38.5	14.3	34.8
Physcion	14.3	60.0		60.0	28.6	42.9	15.4	14.3	27.5
Sterigmatocystin	50.0	10.0	16.7	80.0	14.3	14.3	7.7		23.2
Meleagrin	35.7		50.0	100.0	14.3				20.3
Chaetoglobosin A	28.6	10.0	50.0	40.0			15.4		17.4
Enniatin A1		30.0			14.3	28.6	30.8	14.3	15.9
Stachybotrylactam	7.1		33.3	100.0	14.3				13.0
Alternariolmethylether	7.1				42.9	14.3	7.7		8.7
Alternariol	7.1				28.6	14.3	7.7		7.2
Roquefortine C			33.3	60.0					7.2
Brefeldin A				60.0	14.3				5.8
Kojic acid	7.1	10.0			28.6				5.8
Mycophenolic acid	14.3		16.7			14.3			5.8
Satratoxin G				60.0	14.3				5.8
Satratoxin H				60.0	14.3				5.8
Altenuene		10.0					15.4		4.3
Enniatin A	7.1				28.6				4.3
Ochratoxin A	7.1	20.0							4.3
Viridicatin				20.0	14.3				2.9
Fumiclavine				40.0					2.9
Chanoclavine				40.0					2.9
Alamethicin				20.0					1.4
Apicidin					14.3				1.4
Festuclavin	7.1								1.4
Macrosporin	7.1								1.4
Total no. of different fungal metabolites	17	10	10	16	18	10	11	6	28
Bacterial metabolites									
Chloramphenicol	7.1	20.0	33.3		14.3	71.4	69.2	85.7	37.7
Valinomycin	42.9		33.3	60.0	14.3				17.4
Monactin	7.1		33.3	40.0	14.3				8.7
Nonactin				40.0	14.3				4.3
Staurosporin					14.3				1.4
Total no. of different bacterial metabolites	3	1	3	3	5	1	1	1	5

[a]Man-made mineral fibres (MMF), including mineral wool, stone and glass wool.

air in moisture damage conditions (Brasel et al., 2005b; Gottschalk et al., 2008; Polizzi et al., 2009). The concentrations of metabolites we found in dust and building materials were mostly in the low to mid nanogram per gram range, reaching low microgram per gram levels in a few cases (Table 3). These levels are well comparable to earlier reports on microbial toxin content of indoor dust and building materials (Bloom et al., 2007; Engelhart et al., 2002).

The detection of a multitude of 28 different fungal metabolites in our sample materials confirms earlier reports that have indicated the presence of multiple mycotoxins in moisture-damaged indoor environments (Polizzi et al., 2009; Vishwanath et al., 2009). The results of our exploratory study underline the relevance of using a multi-analyte screening method, which allows to conclude on the spectrum of microbial toxins present indoors. Larsson (2008) has stressed the possible relevance of not only fungal, but also bacterial secondary metabolites as toxic indoor contaminants. While toxic metabolite producing bacterial strains have been isolated from indoor samples (Andersson et al., 1998; Mikkola et al., 2007), actual measurement data on the natural indoor occurrence of these metabolites have so far not been provided. The multi-analyte method applied in our study targeted 27 bacterial metabolites, most of them produced by *Streptomyces* species. Five such compounds were found, with monactin, nonactin, staurosporin, and valinomycin – unlike chloramphenicol – being exclusively detected in building materials, but not house dusts (Table 2).

Streptomyces is a genus of Gram-positive bacteria that commonly occur in soil, but are also known to be present in indoor environments (Hyvärinen et al., 2002; Nevalainen et al., 1991), where they can indicate moisture damage and dampness (Rintala et al., 2004). *Streptomyces* species are also highly potent producers of a large variety of bioactive metabolites (Demain, 1999), such as antibiotics, immunosuppressive agents, enzyme inhibitors, and other pharmacologically active compounds. It is striking to find a toxic metabolite-producing group of bacteria being associated with moisture damage conditions in indoor environments, with some of these metabolites being readily detectable in damaged indoor sample materials.

In this context, it is important to mention that cultivation of building materials did not always predict the presence of the bacterial metabolites in the samples (*data not shown*). The detection of 'dry' actinobacteria-type colonies – a morphology typical for *Streptomyces* and related genera – in culture was not always accompanied by the detection of *Streptomyces* metabolites in the same original material sample and *vice versa*. However, there are several possible explanations for a lack of co-detection of metabolite and producing microbe, including most importantly a still very limited set of bacterial metabolites included in the list of analytes, limitation of cultivation technique to only

Table 3 Levels of microbial metabolites in four moisture-damaged homes: concentrations of microbial metabolites in building materials (BM) and traceability into the respective dust samples

	Microbial metabolites in building materials and dust samples of the same home (maximum concentrations in ng/g building material or dust)														
	Home 2				Home 3				Home 6				Home 9		
	BM	DBD	FD	SD	BM	DBD	FD	SD	BM	DBD	FD	SD	BM	DBD	FD
Altenuene													33		1200
Alternariol	400												13		
Alternariolmethylether	55												1.2		1.3
Apicidin	1300														
Beauvericin			1.4	0.67	0.60	0.32			70		0.75	1.6	0.11	0.05	
Chaetoglobosin A													83		3100
Chloramphenicol		4.8	14	21	14	5.9	17	11	19	5.0	22	42			
Emodin	140	2.4	0.24		78	39	15	84	45	10	5.0		47	12	6.9
Enniatin A	0.83														
Enniatin A1													0.86	2.7	1.0
Enniatin B	0.65	0.75	4.9	0.35	0.78	1.0	4.1	1.1	0.44	0.63	2.4	1.1		9.4	1.6
Equisetin			24			6.4							26	6.1	3.4
Festuclavin	3.3														
Kojic acid													2000		
Macrosporin													120		
Mycophenolic acid	91				88				67						
Ochratoxin A					8.3				32						
Physcion						460							420	46	
Sterigmatocystin									21				110		1.3
Valinomycin					37										
Viridicatin					5.1										

DBD, vacuum cleaner dust bag dust; FD, vacuumed floor dust; SD, settled airborne dust.

viable and culturable microbes and a longer persistence of the microbial metabolites compared to the viability of the producing bacterial cells. In any case, in each home where one or more of the bacterial metabolites were detected and cultivation of building materials was performed, we found also actinobacteria-type colonies in culture, establishing the biological plausibility for the presence of bacterial metabolites in these homes.

The bacterial metabolites present in the sample materials are potent, bioactive substances. Valinomycin, monactin and nonactin are known ionophors that disrupt transmembrane ion gradients. Monactin and nonactin are members of the macrotetrolid antibiotics family and have been shown to modulate cytokine production and T-cell proliferation (Mori et al., 2000; Umland et al., 1999). Research on the biological activity of valinomycin proposed mitochondrial swelling, reduced natural killer cell activity and at higher doses apoptosis in peripheral blood lymphocytes (Paananen et al., 2000). Staurosporin gathers a whole range of biological activities, including anticancer activity by inducing apoptosis in mammalian cells (Stepczynska et al., 2001). Chloramphenicol is an efficient broad-range antibiotic, which because of resistance and safety concerns has been banned for use in food-producing animals and is restricted in human applications. Rare, but severe adverse side effects of chloramphenicol treatment have been reported, such as aplastic anaemia and leukaemia (Rich et al., 1950; Shu et al., 1987).

In addition to bacterial compounds, the multitude of fungal metabolites detected in our study (Table 2) represents an even longer list of different biological activities, which cannot be discussed in detail here. However, it becomes evident that a sound risk assessment on the health implications of indoor microbial toxins is needed. When attempting to assess the health relevance of chronic, low level exposure to microbial metabolites in indoor environments, their variety and co-occurrence as well as the possibility of synergistic effects of different metabolites and other microbial compounds need to be considered. Previous research has shown that the indoor microbes *Stachybotrys chartarum* and *Streptomyces californicus* produce synergistic inflammatory responses in mouse macrophages (Huttunen et al., 2004; Penttinen et al., 2005). Stimulation of the production of toxic secondary metabolites during cocultivation was postulated to be one possible mechanism (Penttinen et al., 2006). Endotoxin – the lipopolysaccharide (LPS) of Gram-negative bacteria – and other microbial compounds are present in all living environments, but are found in elevated levels in moisture damage situations (Garrett et al., 1998; Gorny et al., 2002; Park et al., 2006). The documentation on mycotoxins and LPS acting synergistically in modulating inflammatory processes and causing adverse effects on organ systems is well established (Islam et al., 2007; Kankkunen et al., 2009; Pestka and Zhou, 2006; Zhou et al., 1999).

Emodin, enniatin B, beauvericin and the bacterial toxin chloramphenicol were the most prevalent compounds found in this study and were particularly frequently detected in house dust samples. Preferential detection of certain microbial metabolites in house dusts compared to building material samples could suggest sources other than indoor microbial growth. Plant material and soil, mold-contaminated grain dusts and even insects (Berendsen et al., 2010; Molnár et al., 2010) transferred from outdoors to the indoor environment represent potential sources for low indoor levels of microbial toxins. A recent study from Berendsen et al. (2010) supports the theory that chloramphenicol occurs naturally in soil, produced by common soil bacteria, such as *Streptomyces venezuela*, and accumulates in herbs and grasses. Thus, low indoor levels of chloramphenicol may result from transfer of plant material or dust from out- to indoors. Table 3 summarizes data on the 'traceability' of microbial metabolites in four homes, where both building materials and dust samples were available for analyses. The detection of a given metabolite in damaged building materials along with dust is an indication that microbial growth associated with the moisture damage may be the source of the metabolite. In two of three homes, in which chloramphenicol was found, the analyte was detected not only in dust but also in building material samples. Similarly, the fungal metabolites occurring most frequently in house dust (i.e. beauvericin, emodin, enniatin B) were present also in material samples in the majority of the respective homes, with the exception of equisetin. Nevertheless, uncertainty concerning the sources of microbial toxins detectable indoors suggests conducting sampling and analyses of outdoor air in parallel with indoor assessments in the future.

Limitations of this exploratory study include the relatively small number of samples, which prompted us to present our novel findings on co-occurrence of bacterial and fungal metabolites in a rather descriptive manner. Statistical analyses using microbial toxin data and other exposure and health parameters will be conducted in a next step, once a more complete dataset derived from the HoTeS study will become available. We furthermore aim at concluding on a short-list of the most relevant microbial toxins associated with damage conditions, which will require larger datasets including both sample materials derived from moisture damage and from non-damaged indoor environments.

Sampling and analyses of outdoor air have not been considered in the initial sampling strategy, as this will require more laborious sampling of large air volumes. Based on the study findings, however, we are certainly planning on conducting a systematic study to investi-

gate the outdoor contribution to the presence of microbial metabolites indoors.

Performance characteristics, analytical standards used and other details on the applied multi-analyte HPLC-MS/MS-based methodology have been described previously in a dedicated paper (Vishwanath et al., 2009). As this method was developed as a screening tool for the detection of more than 180 different microbial compounds, there are certain restrictions when it comes to exact quantification. The screening method was shown to be quantitative in case of building materials, however, signal suppression because of matrix effects and non-quantitative extraction impede accurate quantification in dust (Vishwanath et al., 2009). Limits of detection are not consistent for the multitude of target compounds, which may somewhat bias the frequency of detection of certain metabolites. For example, enniatin B and beauvericin show a very high response in the analytical LC-MS/MS methodology, meaning that they are detectable also at very low levels, which could contribute to their relatively high prevalence in the indoor sample materials.

Nevertheless, the applied multi-analyte screening method proved to be an excellent tool to fulfil the objective of assessing the variety of microbial metabolites present in moisture-damaged indoor environments and at creating knowledge on indicative concentrations of the prevalent compounds. The reliability of the multi-analyte HPLC-MS/MS screening method was further confirmed in an interlaboratory method comparison including five building material samples derived from public buildings (Table 1). In addition to the analyses with the multi-analyte screening method, these samples were subjected to analyses with more dedicated methodology targeting a small set of *Stachybotrys* and *Aspergillus* spp. metabolites (Bloom et al., 2007, 2009). The presence of satratoxins G and H and sterigmatocystin in the sample materials – targeted by both methods – was confirmed using the two different approaches.

Conclusions

A whole variety of microbial metabolites is present in sample materials of moisture-damaged indoor environments. Up to 13 different microbial metabolites were found to co-occur in the same home. With the detection of five different metabolites produced by *Streptomyces* species, including chloramphenicol, we provide here for the first time direct proof for the indoor presence of bacterial toxins and their co-occurrence with mycotoxins. Analytical methodology aiming at assessing microbial toxins in indoor samples needs to consider both bacterial and fungal metabolites. Floor dust may be a good sample for an initial assessment of the presence of microbial toxins, as it seems to capture a wide range of indoor metabolites in detectable levels and moreover, is easy to collect.

Several microbial toxins were detectable in settled, previously airborne dust, which indicates that inhalation exposure to these compounds may take place. It remains to be determined whether the detected concentrations of the microbial metabolites are of toxicological relevance or implicate adverse health outcomes. Links to health effects identified among occupants as well as mechanistic, toxicological data on inhalation exposure will help to assess the risk related to indoor exposure to microbial toxins.

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