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Mycobacteria and Fungi in Moisture-Damaged Building Materials

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In contrast to the growth of fungi, the growth of mycobacteria in moisture-damaged building materials has rarely been studied. Environmental mycobacteria were isolated from 23% of samples of moisture-damaged materials (*n* = 88). The occurrence of mycobacteria increased with increasing concentrations of fungi. Mycobacteria may contribute to indoor exposure and associated adverse health effects.

Occupants of moisture-damaged buildings suffer from various respiratory and other health disorders (2, 10). Inhalable components or metabolites of microbes growing in moisture-damaged building materials are considered potential inducers of these effects. Based on available research data, the microbial types that may be responsible for the effects remain unknown (2, 10).

Environmental mycobacteria are heterotrophic organisms that are common in soils, waters, and water distribution systems. They are classified in the genus *Mycobacterium* and order *Actinomycetales* in the class *Actinobacteria*. The genus *Mycobacterium* also includes the *Mycobacterium tuberculosis* complex, the cause of tuberculosis, which does not belong to the group of environmental mycobacteria. The pathogenicity of environmental mycobacteria varies from saprophytes to potential pathogens; thus, the sources of possible infections are always environmental (5). A lipid-rich cell wall makes mycobacterial cells hydrophobic and allows them to tolerate unfavorable environmental conditions, like drought or depletion of nutrients (17). The cell wall of mycobacteria is highly immunogenic, and it is used as an adjuvant in, for example, immunization of laboratory animals (21).

Mycobacterial strains have been isolated from aerosols (18) generated during the dismantling of moisture-damaged buildings. Such strains have induced inflammatory responses, the production of NO and the cytokines interleukin-6 and tumor necrosis factor alpha, in both human and murine cells (6, 7). *M. terrae* has also caused a sustained inflammation in mouse lungs (11). Exposure to mycobacteria may be associated with symptoms with an inflammatory pathway without infection. This conclusion is supported by the finding that exposure to hot tub water (4, 13) and metal-working fluid (14, 20) contaminated with mycobacteria may cause hypersensitivity pneumonitis. Isolation of mycobacteria requires selective methods and long incubation periods. Thus, these organisms are not detected under standard microbial culture conditions and for the most part have been ignored in previous studies (1). To evaluate the possible role of mycobacteria in exposure to organisms in moisture-damaged buildings, we studied the concentrations and diversity of mycobacteria in different types of building materials.

A total of 88 samples of visibly damaged building materials were collected from 34 moisture-damaged buildings. These materials included ceramic products ($n = 36$), wood ($n = 25$), mineral insulation materials ($n = 12$), gypsum board ($n = 4$), and other materials such as paints, plastics, and peat ($n = 11$), as classified previously (8). Thirteen nondamaged samples of the same materials were collected for reference.

Microbes were detached from the materials (0.4 to 5 g) in dilution buffer containing nystatin. Mycobacteria were cultured from the suspensions by direct inoculation onto growth media and after decontamination of 5-ml portions with NaOH plus oxalic acid and cetylpyridinium chloride (12). Identification of mycobacteria was based on methods described previously (18). In addition, partial sequencing of the 16S rRNA gene (22) and the GenoType Mycobacterium CM test (Hain Lifescience GmbH, Nehren, Germany) were used. Fungi were cultured on 2% malt extract (M2) and dichloran glycerol (DG18) media, and heterotrophic bacteria were cultured on tryptone yeast extract glucose agar (8). Data were obtained from 73 (M2 medium) or 75 (DG18 medium and tryptone yeast extract glucose agar) damaged materials and nine reference materials.

Mycobacteria were recovered from 20 of the 88 (23%) damaged building materials at concentrations ranging from 60 CFU/g to 2.0×10^7 CFU/g (median, 1.3×10^3 CFU/g). None of the 13 reference materials yielded mycobacteria ($P > 0.05$, as determined by Fisher's exact test). The isolation frequency for other actinomycetes was 24% (18/75 samples), similar to the values obtained in a previous study (18 to 48%) (8). These results show that contamination of damaged materials with mycobacteria was as frequent as contamination with other actinomycetes but was more frequent than contamination with many fungal genera (8). Only *Penicillium* spp. are usually recovered from more than 20% of samples (8). Previous data on

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TABLE 1. Concentrations of bacteria and fungi in damaged and reference materials

Group	Median concn (range) (CFU/g) in[a]:		P
	Damaged materials (n = 75)	Reference materials (n = 9)	
Actinomycetes	<45 (<45–8.0 × 10^6)	<45 (<45–2.0 × 10^2)	>0.05
Bacteria	1.2 × 10^3 (<45–4.0 × 10^7)	2.3 × 10^2 (<45–1.0 × 10^4)	>0.05
Fungi (M2 agar)[b]	9.5 × 10^2 (<45–4.9 × 10^7)[c]	<45 (<45–4.3 × 10^3)	<0.05
Fungi (DG18 agar)[d]	6.50 × 10^2 (<45–4.3 × 10^7)	<45 (<45–4.7 × 10^3)	0.05

[a] Data were available for 75 damaged and 9 reference materials; for other materials only semiquantitative data were available.
[b] M2 agar, 2% malt extract agar.
[c] n = 73.
[d] DG18 agar, dichloran glycerol agar.

mycobacterial concentrations are scarce, but one observation for moisture-damaged gypsum board liners (10^6 CFU/g) (1, 24) has been reported. Thus, mycobacterial concentrations in building materials can be high.

The materials on which mycobacteria grew were ceramic products (13/36 samples [36%]), wood (5/25 samples [20%]), and mineral insulation materials (2/12 samples [17%]) (P > 0.05, as determined by Kruskal-Wallis one-way analysis of variance). These results indicate that mycobacteria may survive on different surfaces when water is available. Like mycobacterial colonization of water pipes, mycobacteria colonize both organic and inorganic surfaces (15, 19). The high isolation rate for ceramic products indicates that mycobacteria are capable of adapting to alkaline conditions. This was interesting since the optimal pH for growth of many mycobacteria is acidic (9, 16).

Great variation was detected in the concentrations of bacteria and fungi in the damaged materials (Table 1). The occurrence of mycobacteria in building materials increased as the concentration of fungi increased and was more than 30% in samples in which the fungal concentration was 1.0 × 10^4 CFU/g or more (Table 2). The occurrence of mycobacteria was also positively associated with the total concentration of bacteria (median concentrations, 4.3 × 10^6 CFU/g in samples with mycobacteria and 3.2 × 10^2 CFU/g in samples without mycobacteria [P < 0.001, as determined by the Mann-Whitney test]). The presence of mycobacteria was associated with the presence of actinomycetes (P < 0.05), *Aspergillus* spp. (determined with DG18 medium) (P < 0.01), *Fusarium* spp. (determined with M2 and DG18 media) (P < 0.01), and yeasts (determined with M2 and DG18 media) (P < 0.05) (P values determined with Fisher's exact test).

The variety of mycobacterial species among the 179 acid-fast isolates recovered was great (Table 3). The slowly growing species *M. intracellulare* and *M. tusciae* and the rapidly growing species *M. chelonae*, *M. fortuitum*, *M. mucogenicum*, and *M. septicum* are potential pathogens causing pulmonary or skin and other soft tissue infections (3, 5, 23). Some species detected, including *M. intracellulare*, *M. terrae*, and *M. fortuitum*, have previously been isolated from aerosols during the remediation of moisture-damaged buildings (18). The isolation of unknown mycobacteria was expected since building materials

TABLE 2. Occurrence of mycobacteria in building materials with different concentrations of fungi

Medium	No. of samples positive for mycobacteria/ no. tested (%) with the following concn of fungi			P
	<45 CFU/g	45–9.99 × 10^3 CFU/g	≥1.0 × 10^4 CFU/g	
M2 agar[a]	3/30 (10)	5/22 (23)	11/30 (37)	<0.05
DG18 agar[b]	3/33 (9)	6/23 (26)	10/28 (36)	<0.05

[a] M2 agar, 2% malt extract agar.
[b] DG18 agar, dichloran glycerol agar.

TABLE 3. Mycobacterial species isolated from different moisture-damaged building materials

Material[a]	Species or strain[b]	No. of samples
Ceramic products (13/36 samples [36%])	*M. intracellulare*	3
	M. gordonae	3
	SG2	2
	SG3	2
	M. tusciae	2
	M. doricum	1
	Mycobacterium sp. strain MCRO 6	1
	M. terrae	1
	M. tokaiense	1
	SG1	1
	M. chelonae	1
	M. flavescens	1
	M. fortuitum	1
	M. gilvum	1
	M. mucogenicum	1
	M. septicum	1
Wood (5/25 samples [20%])	*M. intracellulare*	2
	Mycobacterium sp. strain MCRO 6	2
	M. terrae	1
	SG1	1
	SG3	1
	M. fortuitum	1
	M. tokaiense	1
Mineral insulation materials (2/12 samples [17%])	*M. vaccae*	1
	RG1	1
	RG2	1
	SG3	1
	SG4	1

[a] The number (percent) of samples from which mycobacterial species were isolated is shown in parentheses.
[b] RG1 and RG2 are unidentified chromogenic rapidly growing mycobacteria; SG1 and SG3 are unidentified nonchromogenic slowly growing mycobacteria; and SG2 and SG4 are unidentified scotochromogenic slowly growing mycobacteria.

have been poorly explored for mycobacteria. Ceramic products yielded the greatest variety of species, but there was no difference between the material groups in terms of the number of mycobacterial species per sample (2.1, 1.8, and 2.5 species per sample for ceramic, wood, and mineral insulation materials, respectively; $P > 0.05$, as determined by Kruskal-Wallis one-way analysis of variance).

In conclusion, mycobacteria were common in moisture-damaged building materials, and their occurrence increased with the increasing degree of mold damage. These results, combined with previous data on aerosolization during dismantling and the ability of mycobacteria to cause inflammatory responses, suggest that these organisms may contribute to the adverse health effects associated with moisture-damaged buildings.

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