

Amoebae and other protozoa in moisture-damaged building materials

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ABSTRACT

Mould growth in damp buildings has been shown to be associated with adverse health symptoms. The fungal and bacterial growth of the damp buildings has been studied, but little attention has been paid on other organisms amplifying in the moisture-damaged materials. We examined moist building materials for protozoa, concentrating on amoebae. Material samples ($n = 124$) from moisture-damaged buildings were analysed for amoebae, fungi and bacteria. Amoebae were found in 22% of the samples, and they were found to favour co-occurrence with bacteria and *Acremonium* spp., *Aspergillus versicolor*, *Chaetomium* spp. and *Trichoderma* spp. In addition, 11 seriously damaged samples were screened for other protozoa. Ciliates and flagellates were found in almost every sample analysed. As amoebae are known to host pathogenic bacteria, they may have a role in the network of exposure and health effects associated with the moisture-damaged buildings.

INDEX TERMS

Amoebae; Moisture-damaged buildings; Fungi; Bacteria

INTRODUCTION

Moisture and mould growth in buildings have been clearly shown to be in association with several types of adverse health reactions (Verhoeff and Burge, 1997; Peat *et al.*, 1998). The exact causative agents remain, however, yet unknown. The fungal and bacterial contamination of the damp buildings has been studied at length, but little attention has been paid on the other organisms inhabiting the moisture-damaged materials.

We investigated what other kinds of organisms apart from fungi and bacteria could be found in moist building materials, concentrating on the protozoa, especially amoebae. Apart from the pathogenicity of several species of amoebae and other protozoa, amoebae can also act as host cells to pathogenic bacteria, such as *Legionellae* and *Chlamydiae* (Rowbotham, 1980; Essig *et al.*, 1997). Therefore, amoebae can create a favourable environment for bacteria otherwise unable to survive in the building materials.

The aim of this study was to investigate the occurrence of amoebae and other protozoans in moisture-damaged building materials.

METHODS

We analysed 124 material samples from moisture-damaged buildings for amoebae, fungi and bacteria. In addition, 11 severely damaged samples were screened for other protozoa and nematodes. The presence of amoebae was determined after a method developed by Newsome *et al.* (1998): a non-nutrient agar plate was streaked with heat-killed *Escherichia* (*E.*) *coli* in X configuration. A piece of the sample was placed in the centre of *E. coli* lines, and the samples were incubated for 72 h at $25 \pm 2^\circ\text{C}$. After the incubation plates were examined microscopically for amoebae, other protozoa were analysed similarly to amoebae but without

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E. coli lines and ca. 0.5 ml of sterile deionized water was added on top of the sample to aid the movement of protozoa. Fungal and bacterial analyses were performed either by direct ($n = 75$) or dilution ($n = 49$) plating, as previously described (Hyvärinen *et al.*, 2002; Reiman *et al.*, 1999). The co-occurrence and association of amounts for amoebae with fungi and bacteria were analysed with SPSS version 10.1.3.

RESULTS

We found amoebae in 27 samples ($\approx 22\%$) out of 124. Amoebae were more likely to occur if actinomycetes or other bacteria, or the fungi *Acremonium* spp., *Aspergillus versicolor*, *Chaetomium* spp. or *Trichoderma* spp. were present. Distributions of samples with or without amoebae for some individual microbes or groups are presented in Figure 1.

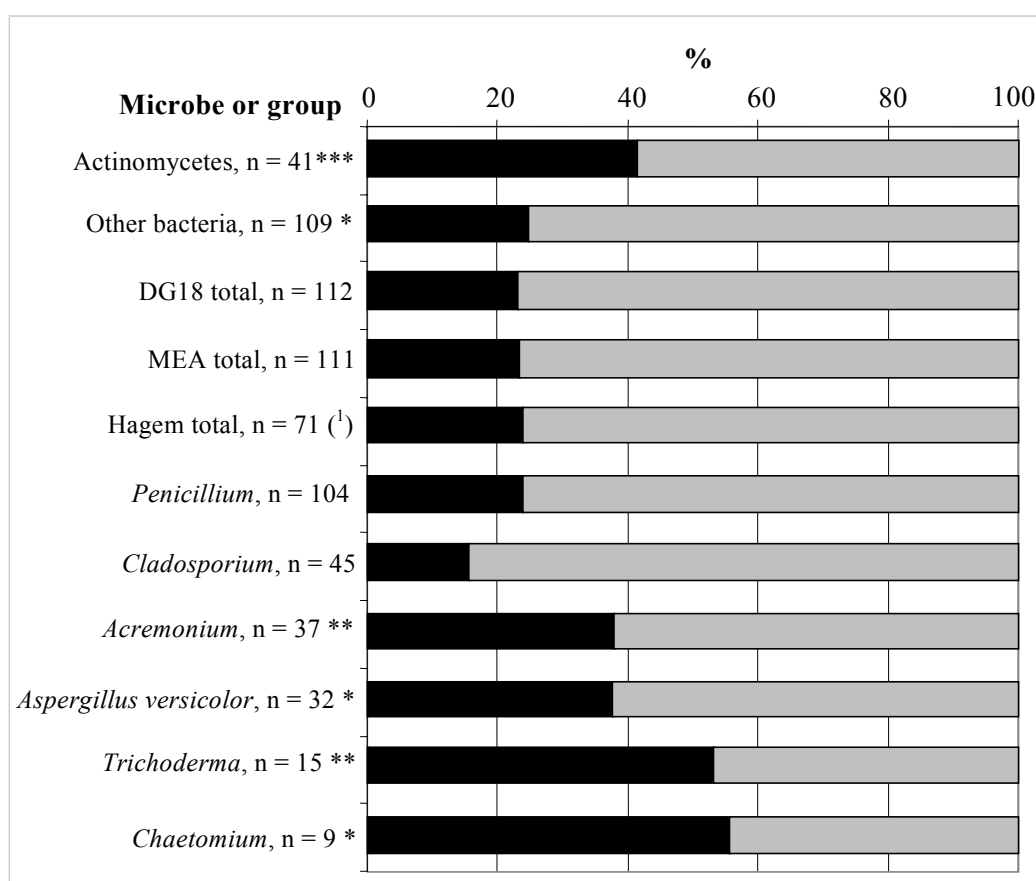


Figure 1 The percentage of samples of the microbe or group stated occurring with amoebae (■) or without amoebae (□). *** = The association of amoebae and microbe stated is significant at $p < 0.001$ level, ** = $p < 0.01$, * = $p < 0.05$, (¹) used only with direct plating method.

The amount of amoebae was associated with the amount of bacteria, actinomycetes and total fungal counts on DG18 and MEA plates. The amounts of *Penicillium* spp., *Paecilomyces* spp., Sphaeropsidales and *Stachybotrys* spp. on either DG18 or MEA were associated with the number of amoebae. Ciliates and flagellates were found in almost every sample analysed (73 and 91 % of the 11 samples, respectively). In a single, seriously damaged sample, also nematodes were detected.

DISCUSSION

Amoebae seemed to favour co-occurrence with some microbes that are considered as indicators for moisture damage in building, such as fungi *Aspergillus versicolor*, *Stachybotrys*

spp. and *Trichoderma* spp. and actinomycetes. These microbes often occur when the water activity of the material is high ($a_w > 0.85$) (Samson *et al.*, 1994). As amoebae also favour moist environments (Storer *et al.*, 1979), the co-occurrence and association of amoebae with the stated microbes could at least in part be a result of their similar moisture requirements. One explanation for the association can also be that amoebae feed on bacteria and fungi, among other small organic particles.

Several species of free-living amoebae (especially *Acanthamoeba* and *Hartmanella*) are known to harbour bacteria as natural endosymbionts. These bacteria include, e.g. *Legionella* spp. and *Rickettsia*-like bacteria (Newsome *et al.*, 1998, Fritsche *et al.*, 1999). Several other bacteria have been shown *in vitro* to be able to survive and replicate inside amoebae, such as species of *Chlamydia*, *Listeria*, *Mycobacterium* and coliforms (Essig *et al.*, 1997; Ly and Muller, 1990; Cirillo *et al.*, 1997; King *et al.*, 1988). As some of the bacteria living in amoebae are human pathogens, amoebae might have a role in the complex network of microbial exposure and health symptoms associated with moisture-damaged buildings.

Based on these preliminary results, ciliates and flagellates are also quite common in the severely damaged building materials. As only 11 samples were analysed, however, definite conclusions should not be made about the occurrence of ciliates and flagellates in the building environment or their possible role as exposing agents in the indoor environment.

CONCLUSION AND IMPLICATIONS

Amoebae and other protozoans seem to be rather common in moisture-damaged building materials. Further studies are needed to establish their role as exposing agents or causative agents of health symptoms in moisture-damaged buildings.

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