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Detection of bacterial cytotoxic activities from water-damaged ceiling tile material following incubation on blood agar

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Samples of ceiling tiles with high levels of bacteria exhibited cytotoxic activities on a HEP-2 tissue culture assay. Ceiling tiles containing low levels of bacterial colonization did not show cytotoxic activities on the HEP-2 tissue culture assay. Using a spread plate procedure on blood agar plates, the levels of bacteria colonizing portions of cellulosic indoor ceiling tiles were easily identified. Levels of bacteria measured by this simple procedure may be a good indicator of microbial colonization of indoor building materials especially in the case of water damage. We suggest that bacterial levels above 150 CFU g^{-1} of ceiling tile material indicate colonization has occurred.

Keywords: ceiling tiles; bacteria; cytotoxicity; water damage

Introduction

There is a growing amount of data in the scientific literature concerning origins of pollutants and allergens from indoor sources [8]. Water damage to building materials facilitates the growth of microorganisms in a wide variety of indoor environments. Previous studies with cellulosic ceiling tile materials suggest that water damage promotes the growth of microorganisms which are capable of releasing toxic and immunoreactive agents [1,7]. The release of bioactive agents may be involved in the etiology of health symptoms associated with certain types of indoor air quality deterioration and increases in respiratory symptoms have been associated with household water damage [4].

There is a need for developing procedures that will quickly and conveniently determine the effects of water damage on microbial colonization of indoor materials such as ceiling tiles [6]. Using a simple procedure for detecting numbers of bacteria in ceiling tile material, we were able to identify quickly those portions of ceiling tiles that had experienced bacterial colonization even when there were no obvious 'water stain' residual marks. The mixed bacterial communities cultured '*in situ*' from ceiling tile material were subsequently tested for cytotoxic activities.

Materials and methods

Sampling of ceiling tiles

Five ceiling tiles (with obvious water damage stains covering approximately 10–20% of each tile) were sampled from the first floor of the five-story, 25-year-old Natural Science Building on the campus of Northern Kentucky University. Five years prior to the sampling, the ventilation system of this building had been inspected and approved as meeting industry standards. Because the ceiling tiles had been replaced at that time, water damage to ceiling tiles had occurred within the previous 5 years.

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Individual ceiling tiles were taken down and labeled on the backsides for future reference. Sampling was done by scraping material from the backside of each tile with a sterile, serrated knife. A sample of material was taken from the center of an area where there was apparent water damage, as evidenced by residual staining. A second sample was also taken from the same tile in an area 30 cm from the edge of the original stained area. This second sample was taken from an area where there was no apparent water damage. A total of 10 g of material was obtained from each site and stored in sterile plastic bags. The ceiling tiles were replaced in their original locations after sampling.

Testing for microbial growth

Twenty-five milligrams of each sample were distributed onto the surface of the following media by simply sprinkling the material across each plate. The media were incubated as follows: blood agar (BAP), 35°C for 48 h; Sabouraud's dextrose agar (SAB), 25°C for 5 days; and trypticase soy agar (TSA), 25°C for 5 days.

Cytotoxicity testing

Twenty-four hours prior to the cytotoxicity assay, 1.0 g of each ceiling tile sample was placed into a separate, sterile tube and 3.0 ml of sterile tap water was added to each tube. Next, 0.5 g of the damp ceiling tile material was immediately placed directly onto a nitrocellulose membrane which had been placed on the surface of a blood agar plate. The plate was then incubated for 24 h at 35°C. For the cytotoxicity assay, the membrane filter (with the plug material on the surface after incubation) was transferred onto Hep-2 cells using the procedure described by Lye and Dufour [3] for assaying cytotoxicities of bacteria commonly found in water.

Results

Table 1 shows the numbers of total bacteria cultured from the ceiling tile samples. The culture of ceiling tile material on blood agar yielded the highest counts among the three different media tested. Blood agar is an attractive medium

Table 1 Bacterial colony forming units in 25-mg samples of ceiling tile material

Medium	CFU from 25-mg ceiling tile sample									
	1[a]	1H[b]	2	2H	3	3H	4	4H	5	5H
BAP	2	24	8	1050	0	1	0	4	4	673
TSA	2	21	8	540	3	8	0	1	3	420
SAB	0	3	1	290	0	0	0	1	2	39
Cytotoxicity[c]	neg	pos	neg	pos	neg	neg	neg	pos	neg	pos

[a]Nondamaged portion of ceiling tile.
[b]'H' refers to water-damaged portion of same ceiling tile.
[c]Measured by noting discrete areas of cellular destruction after 16 h exposure.

to use in the isolation of cytotoxic bacteria because it has been shown to be the best enrichment procedure for these types of bacteria [2,5].

Ceiling tile No. 1 exhibited cytotoxic activity only from the water-damaged area which contained 960 CFU g^{-1} of material. Material from a nonstained area of this same tile did not exhibit cytotoxic activity and contained tenfold less bacteria at only 80 CFU g^{-1} of material.

Ceiling tile No. 2 exhibited cytotoxic activity only from the water-damaged area which contained the highest level of bacterial colonization of all of the tiles sampled, 42 000 CFU g^{-1} of material. Material from a nonstained area of this tile did not exhibit cytotoxic activity but did contain 320 CFU g^{-1} of material. These results suggest that portions of ceiling tiles that have extensive bacterial colonization (such as tile No. 2) will have increased numbers of bacteria in the nonstained areas as a result of a concentration gradient effect from the heavily colonized, water-damaged areas.

Ceiling tile No. 3 did not exhibit cytotoxic activity from the water-damaged area. The bacterial numbers associated with the water-damaged area were 40 CFU g^{-1}. There were no bacteria isolated from the nondamaged area. These results suggest that even though a water stain may be visible on ceiling tiles, bacterial colonization may not have occurred.

Ceiling tile No. 4 exhibited cytotoxicity from the water-damaged area even though the bacterial levels were low compared to the other positive tile samples, 160 CFU g^{-1}. There were no bacteria isolated from the nondamaged area of this tile.

Ceiling tile No. 5 exhibited cytotoxic activity from a heavily colonized area of water damage, 14 920 CFU g^{-1} of material. No cytotoxic activity was seen from a nondamaged area of the tile.

Discussion

This survey was initially designed to detect bacterial colonization of ceiling tiles. The bacterial levels associated with water-damaged portions of cellulosic ceiling tiles have proven to be easily detected with the procedure described in this article. Although the presence of 'water stains' was not always indicative of increased bacterial numbers, water-damaged areas of a single ceiling often gave higher bacterial numbers than nondamaged areas of the same tile. High levels of bacteria are not commonly found on nondamaged ceiling tiles.

Levels of bacteria measured by our simple procedure may be a good indicator of microbial colonization of building materials especially in the case of water damage. Our results revealed that background levels of bacteria in nondamaged areas of ceiling tiles typically ranged from zero to 320 CFU g^{-1} of material. The upper limits of nondamaged areas of ceiling tiles appeared to be influenced by how heavily the colonization was in the damaged portions of that same tile.

The second point of our survey addresses the potential of ceiling tiles heavily colonized with bacteria to contribute to the release of cytotoxic chemicals if water damage reoccurs. Levels of bacteria cultured from water-stained areas of ceiling tiles ranged from 40 to 42 000 CFU g^{-1} of material. Levels of bacteria below 150 CFU g^{-1} in water-stained areas did not yield cytotoxic activities from our tissue assays even though there was apparent water damage. However, levels of bacterial colonization above 150 CFU per gram of material were associated with cytotoxic activity. We suggest that bacterial levels below 150 CFU g^{-1} of ceiling tile material be used to indicate non-colonized ceiling tiles with low levels of possible cytotoxic activities. Levels above 150 CFU g^{-1} indicate that colonization of the ceiling tiles by bacteria has occurred irrespective of the presence of visible water stains. Colonization of ceiling tiles by increased numbers of bacteria may result in increased numbers of those bacteria with cytotoxic activities. Current studies are underway to further identify and characterize the cytotoxic Gram-positive rods and cocci that are most commonly isolated from ceiling tile material.

The ability to determine conveniently the bacterial colonization levels of ceiling tile materials within 24 h is a valuable method for assessing the extent of water damage in individual ceiling tiles. This method will allow more precise monitoring of the extent and ramifications of water damage effects in ceiling material. In situations where water damage is suspected to be contributing to indoor pollution, this technique may prove useful for replacement and maintenance of low quality ceiling tile materials.

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