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Journal of Microbiological Methods 61 (2005) 343–347
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Method for evaluating mold growth on ceiling tile

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Received 23 November 2004; received in revised form 15 December 2004; accepted 15 December 2004
Available online 25 January 2005

Abstract

A method to extract mold spores from porous ceiling tiles was developed using a masticator blender. Ceiling tiles were inoculated and analyzed using four species of mold. Statistical analysis comparing results obtained by masticator extraction and the swab method was performed. The masticator method was demonstrated as efficient for bulk sampling of ceiling tiles.

Keywords: Mold; Masticator; Ceiling tile

1. Introduction

The collection of surface samples for mold and bacteria contaminants often employs sterile cotton swabs, contact agar plates and adhesive tapes (Dillon et al., 1996; Macher, 1999; Menetrez et al., 2002). These methods are particularly useful for testing the presence of biocontaminants on non-porous surfaces with minimal harm to the material sampled. Quantification of biocontaminant populations can be accomplished using the swab method combined with serial dilutions. However for more exact bulk surface sampling, other methods have been developed (Chang et al., 1995; Menetrez et al., 2002, 2004). It has been shown that suspending bulk samples in phosphate buffered saline followed by agitation and serial dilutions yields better estimates of the mold population (Macher, 1999).

A method widely used for the microbiological sample extraction from a wide range of porous and non-porous materials is the homogenization of the sample using a masticator blender (MB) or stomacher. With the MB method, samples are suspended in a solution of phosphate buffered saline and agitated in a blender to separate the microorganisms on surfaces and/or embedded in the material. The MB method is used for the microbial analysis of food (Odumeru et al., 2004; Purvis et al., 1987), biosolids (Method 1682, 1998), biofilms (Ammor et al., 2004), and tissue biopsies (Liebana et al., 1998).

The objective of this study was to develop and evaluate a bulk sample test method for porous ceiling

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doi:10.1016/j.mimet.2004.12.013

tiles to detect and quantify mold growth using a masticator blender.

Results were compared to those obtained with the swab method. Statistical analysis showed that the masticator method yielded higher estimates of the mold population.

2. Materials and methods

2.1. Ceiling tile

Ceiling tiles that had been in use for approximately 1 year in a laboratory were made available due to construction changes. These were Class A, standard-white, fire-retardant, textured-faced ceiling tiles composed of wood fiber (0–60%) and fibrous glass (0–13%) as specified by the manufacturer. The material was cut into sample sizes measuring 3×1.5 in. (7.62×3.81 cm). The white surface (portion facing the room) was then equally divided into four sections 1.5×0.75 in. (3.81×1.905 cm) each, by scaring the surface of the sample tile with a razor blade. The divisions provided a visual guide for the inoculation and analysis procedures. Each piece of ceiling tile was then individually wrapped in aluminum foil and steam sterilized by autoclaving.

2.2. Fungal cultures

Four mold cultures isolated from water-damaged buildings were provided by the Research Triangle Institute, North Carolina: *Aspergillus versicolor* (RTI 3843), *Cladosporium sphaerospermum* (RTI 3491), *Penicillium brevicompactum* (RTI 3791), and *Stachybotrys chartarum* (RTI 3441). All mold cultures were grown on Sabouraud Dextrose Agar from 7–14 days. Spore suspensions were prepared using the method described in Crow et al. (1994) with modifications for harvesting mold spores (Dean et al., 2004). The final spore concentration for each mold suspension was approximately 10^6 spores/mL.

2.3. Substrate inoculation

A total of 12 pieces of ceiling tile were inoculated with mold spores at the starting date. Each ceiling tile was placed on a sterile 15 mm Petri dish and wetted with 9 mL of sterile distilled water. Spore suspensions of 0.1 mL were pipetted to each of the four sections within the 3×1.5 in. (7.62×3.81 cm) piece to a final concentration of 10^5 spores/mL. Each of the four molds (*Stachybotrys chartarum*, *Cladosporium sphaerospermum*, *Penicillium brevicompactum*, *Aspergillus versicolor*) was used to inoculate three ceiling tiles. Additionally, three uninoculated sterilized and unsterilized ceiling tiles were not inoculated to serve as controls.

The Petri dishes were incubated in the dark inside a static microbial growth chamber at 21.0 °C and 100% RH (Chang et al., 1995; Foarde et al., 1996).

Four ceiling tiles, each with a different mold, were analyzed each sampling date along with a positive and a negative control. Samples were analyzed after 14, 21 and 28 days of incubation. A 1.5×0.75 in. (3.81×1.905 cm) section was analyzed using the swab method and another 1.5×0.75 in. (3.81×1.905 cm) section was analyzed using the masticator blender method. The remaining two sections were discarded.

3. Masticator blender method

In order to achieve statistically reliable counts/plate (30–300 colonies/plate) the sample dilution used was dependent on the amount of growth observed on the surface of the ceiling tile. PBT consisting of 0.01 M phosphate buffer with 0.05% (v/v) Tween 20 (Sigma Chemical, St. Louis, MO, USA) was used to dilute the sample. For light to moderate growth a 1.5×0.75 in. (3.81×1.905 cm) rectangular section was aseptically cut, weighed for consistency (1.0 g) and transferred to a sterile polyethylene bag, with 9.0 mL of sterile PBT, to prepare a 1/10 dilution of the sample. For heavy growth, 99 ml of sterile PBT was used to prepare a 1/100 dilution sample. The bag with sample was inserted in a Nasco masticator blender (Nasco Sampling Products, Modesto, CA, USA) and homogenized for 15 s at 10 beats per second. The homogenate was then diluted as needed (between 10^{-5} and 10^{-7}), and plated in duplicate on Sabouraud Dextrose Agar (SDA). The SDA plates were incubated at room temperature and colony-forming units (CFU) were determined when growth was observed.

4. Swab method

The swab method with modifications published by Dillon et al., 1996 was used. Sterile seven-inch cotton-tipped swabs were used for extracting spores from the 1.5×0.75 in. (3.81×1.905 cm) sections of ceiling tile. The sterile swab was wetted in PBT and used to swab the whole sample surface once. The swabs were then soaked from 1 to 2 min in 1.0 mL of PBT. After vigorously mixing the suspension, it was diluted as necessary (between 10^{-5} and 10^{-7}) and then plated in duplicate on SDA. CFU's were determined as described above.

5. Results and discussion

Comparison of the swab method and MB method for the recovery of mold spores from ceiling tiles was performed. Individually inoculated samples with mold spores of *S. chartarum*, *C. sphaerospermum*, *P. brevicompactum* and *A. versicolor*, were analyzed for their mold population after 14, 21 and 28 days of incubation.

Table 1 and Fig. 1 compare the CFU's for both methods. All of the MB samples and two of the swab method samples showed results indicating an increase in the number of spores from an initial inoculum of 10^5 spores/mL to a range of approximately 10^6–10^8 spores/mL after 14 days of incubation. Of the remaining two swab method samples moderate growth of 8×10^5 CFU's was observed by *P. brevicompactum*, and a decrease in CFU's for *C. sphaerospermum* was observed below which could be accurately counted.

After 14 days, MB sample populations stayed at a relatively stable level. Spore populations of approximately 10^7 CFU's for *S. chartarum* and *C. sphaerospermum* were counted. A reduction of one log was observed for *P. brevicompactum* from 10^7 at 14 d to 10^6 at 21 d and for *A. versicolor* from 10^8 at 21 d to 10^7 at 28 d. However, swab method counts were consistently lower than MB counts without exception.

No growth was observed on the sterile, uninoculated controls. The unsterilized, uninoculated tiles showed occasional contamination with two genera (*Penicillium* sp. and *Aspergillus* sp.) that were not observed in the sterile, uninoculated tiles. Thus, indicating that sterilization of the ceiling tiles prior to inoculation eliminated all endogenous background mold.

Table 1
Comparison of colony forming units (CFU) between "swab" method vs. "masticator blender" method

Sample	CFU w "swab" method	CFU w "masticator blender" method
Stachybotrys chartarum (Sc)		
14 days	1.0×10^7	3.0×10^7
21 days	5.7×10^6	2.9×10^7
28 days	8.1×10^6	3.4×10^7
Cladosporium sphaerospermum (Cs)		
14 days	ND	2.8×10^7
21 days	3.1×10^6	5.3×10^7
28 days	4.5×10^6	3.5×10^7
Penicillium brevicompactum (Pb)		
14 days	8.0×10^5	1.5×10^7
21 days	6.0×10^6	7.7×10^6
28 days	1.2×10^6	1.3×10^7
Aspergillus versicolor (Av)		
14 days	5.0×10^7	1.1×10^8
21 days	4.5×10^7	1.1×10^8
28 days	1.3×10^7	5.3×10^7
Controls—sterile		
14 days	No growth (NG)	No growth (NG)
21 days	No growth (NG)	No growth (NG)
28 days	No growth (NG)	No growth (NG)
Controls—not sterile		
14 days	Growth (G)	Not tested (NT)
21 days	Growth (G)	Not tested (NT)
28 days	Growth (G)	Not tested (NT)

Results showed that the CFU's for each mold variety tested were higher using the MB method. The agitation and blending action provided by the masticator blender was able to improve separation of the mold spores from the surfaces and pores of the ceiling tile samples. The increased CFU counts using the MB method is consistent with bulk sampling studies that show the suspension of samples in buffer and agitation effectively separate microbial growth deeply embedded in a sample (Ammor et al., 2004; Liebana et al., 1998; Method 1682, 1998; Odumeru et al., 2004; Purvis et al., 1987).

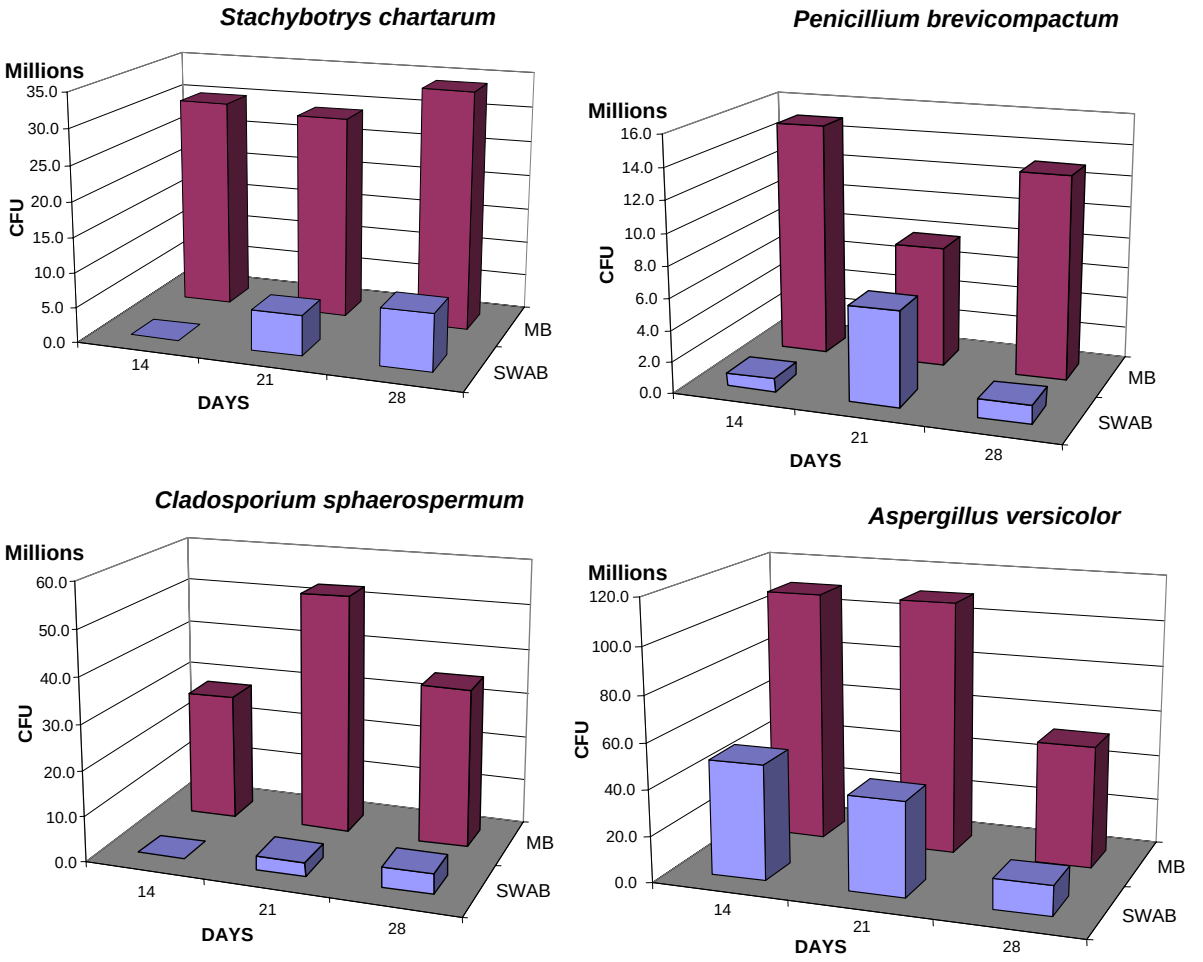

Fig. 1. Comparison of CFU between swab and MB method.

Table 2 lists the means and standard deviations of the normalized ratio of CFU counts taken from Table 1, for each mold by the spore extraction method used. CFU's were transformed to "counts" by dividing each value by 10^6 before performing the statistical analysis. The results of the tests confirm that the overall mean of the MB values is significantly greater than the overall mean of the swab values. Consistent with overall greater num-

Table 2
Summary of means and standard deviations

Mold	Mean		Standard deviation	
	Swab	MB	Swab	MB
Stachybotrys chartarum	7.93	31.00	2.15	2.65
Cladosporium sphaerospermum	3.80	38.67	0.99	12.90
Penicillium brevicompactum	2.67	11.90	2.89	3.77
Aspergillus versicolor	36.00	91.00	20.08	32.91

The data were also transformed by taking base-10 logarithms of CFU. The results—not reported here—are similar to those for the counts (i.e. CFU/10^6) analyzed in this report.
CFU were transformed by dividing each value by 10^6 before performing any analysis.

Table 3
Comparison of proportionality between MB and swab methods

	Mean MB/swab ratio	Standard deviation MB/swab ratio
Stachybotrys chartarum	3.9	1.2
Cladosporium sphaerospermum	10.2	13.0
Penicillium brevicompactum	4.5	1.3
Aspergillus versicolor	2.5	1.6

bers of recovered CFU's, the MB values are greater in variability than that of the swab values.

A comparison of proportionality (Table 3) can be made in which the mean and standard deviation CFU values for swab sampling are divided into the MB values listed in Table 2. Table 3 lists that the ratio of standard deviations were less than the ratio of mean values for *S. chartarum*, *P. brevicompactum* and *A. versicolor*. This indicates that for these organisms, variability was proportionally less for the MB method. The ratio of standard deviations for *C. sphaerospermum* was marginally greater than the ratio of mean values. This is not believed to be significant and may be due to sample size.

The occurrence of sampling variability is supported by Foarde et al. (1993) that demonstrated growth of *P. glabrum* and *A. versicolor* to be influenced by the moisture content and the type of ceiling tile as well as the RH and temperature in the incubation chamber. However, environmental factors associated with the propagation of mold were beyond the scope of this study and statistical analysis.

This study shows that homogenizing samples of bulk ceiling tiles with a masticator blender is an effective method to recover mold spores from porous surfaces. Additionally, the results confirm that bulk sampling using agitation yield higher estimates of mold populations. Continued analysis employing the MB method with other bulk building materials is being studied for its application to antimicrobial efficacy testing.

Acknowledgements

The authors greatly appreciate the assistance of Dr. Ross Leadbetter and Kevin J. Ross from the University of North Carolina at Chapel Hill for the statistical analysis.

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