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Ergosterol Content in Various Fungal Species and Biocontaminated Building Materials

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This paper reports the ergosterol content for microbial cultures of six filamentous fungi, three yeast species, and one actinomycete and the ergosterol levels in 40 samples of building materials (wood chip, gypsum board, and glass wool) contaminated by microorganisms. The samples were hydrolyzed in alkaline methanol, and sterols were silylated and analyzed by gas chromatography-mass spectrometry. The average ergosterol content varied widely among the fungal species over the range of 2.6 to 42 μg/ml of dry mass or 0.00011 to 17 pg/spore or cell. Ergosterol could not be detected in the actinomycete culture. The results for both the fungal cultures and building material samples supported the idea that the ergosterol content reflects the concentration of filamentous fungi but it underestimates the occurrence of yeast cells. The ergosterol content in building material samples ranged from 0.017 to 68 μg/g of dry mass of material. A good agreement between the ergosterol concentration and viable fungal concentrations was detected in the wood chip ($r > 0.66$, $P \leq 0.009$) and gypsum board samples ($r > 0.48$, $P \leq 0.059$), whereas no relationship between these factors was observed in the glass wool samples. For the pooled data of the building materials, the ergosterol content correlated significantly with the viable fungal levels ($r > 0.63$, $P < 0.0001$). In conclusion, the ergosterol concentration could be a suitable marker for estimation of fungal concentrations in contaminated building materials with certain reservations, including the underestimation of yeast concentrations.

Ergosterol is the primary sterol in the cell membranes of filamentous fungi and is either absent or a minor component in most higher plants. It is also present in membranes in the yeast cell wall and mitochondria (1, 9). Ergosterol is a constituent of membranes in mycelia, spores, and vegetative cells (12). Ergosterol content has been widely used as an estimate of fungal biomass in various environments, e.g., in soil and aquatic systems, because a strong correlation has been found between ergosterol content and fungal dry mass (8, 12, 13, 17, 19). However, the amount of ergosterol in fungal tissue is not constant. There are interactions between the amount of ergosterol and fungal species, age of the culture, developmental stage (growth phase, hyphal formation, and sporulation), and growth conditions (growth media, pH, and temperature), although no clear trend for the ergosterol content in any of these factors has yet been detected (6, 13, 17). In hyphomycetes and ascomycetes, ergosterol concentrations ranging from 2.3 to 11.9 μg of ergosterol/mg of dry mycelium have been reported (6, 13), and the ergosterol content for *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, *Cladosporium*, *Candida*, and *Alternaria* species has ranged from 0.4 to 14.3 μg/mg (1, 17, 18). A relationship between the amount of ergosterol per spore and spore size has also been suggested. In *Aspergillus*, *Penicillium*, and *Cladosporium* species, the average ergosterol content per spore has been reported to range from 1.7 to 5.1 pg/spore, with a wide interspecies variability (11). However, the ergosterol contents in spores seem to be quite similar for those fungal species studied when adjustments are made for spore volume and surface area.

Ergosterol has also been suggested for use in quantifying fungal growth in solid substrates because of a good correlation between the ergosterol content and hyphal length (17). Recently, ergosterol measurements were proposed as a new method for determination of total fungal biomass in investigations of indoor environments (4, 11). In house dust, ergosterol concentrations of 0.7 to 45 μg/g have been reported (1, 10, 16), and ergosterol concentrations in indoor air within the range of 0.01 to 194 ng/m^3 have also been measured successfully (11).

In this study, the ergosterol contents of six filamentous fungal species, three yeast species, and one actinomycete species were measured in pure broth cultures. The ergosterol concentration was also determined for building material samples contaminated by fungal and actinomycete growth. The objective of this study was to produce new information on the variation of the ergosterol content between different fungi, including yeasts, and to evaluate the suitability of ergosterol content as a marker of fungal growth in water-damaged building materials.

MATERIALS AND METHODS

Microbial strains. The following microbial strains were used: *Acremonium furcatum* UKU (University of Kuopio) 1, isolated from water-damaged wood; *Aspergillus versicolor* UKU 3, isolated from water-damaged wallpaper; *Aureobasidium pullulans* DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) 62074; *Cladosporium cladosporioides* DSM 62121; *Cryptococcus albidus* UKU 17, isolated from water-damaged particle board; *Penicillium brevicompactum* ATCC 58606; *Rhodotorula minuta* UKU 9, *Rhodotorula mucilaginosa* UKU 16, and *Stachybotrys chartarum* UKU 10, all three isolated from water-damaged gypsum board; and *Streptomyces californicus* A 12 (National Public Health Institute, Kuopio, Finland), isolated from a damp building. The fungal strains were first cultivated on 2% malt extract agar medium (Biokar Diagnostics, Beauvais, France) for 7 to 10 days, and *Streptomyces californicus* was cultivated on tryptone-yeast extract-glucose agar (Oxoid Ltd., Basingstoke, England) for 14 days at 25°C in the dark. Cultures were suspended in 4 to 5 ml of dilution water (42.5 mg of KH_2PO_4, 250 mg of $MgSO_4 \cdot 7H_2O$, 8 mg of NaOH, 0.02% Tween 80 detergent, each per 1 liter of deionized water) by flooding the agar plate, gently stirring the solution with a sterile glass rod, and transferring it to test tubes. One milliliter of each fungal suspension was inoculated in 50 ml of 2% malt extract broth (Biokar). A suspension from *Streptomyces californicus* cultures was inoculated in 50 ml of tryptone-yeast extract-glucose broth (Oxoid Ltd.). The

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TABLE 1. Ergosterol content of the different fungi tested

Fungal species	Spore or cell dimensions (μm)	Spores (10^6)/mg of fungal dry mass	Ergosterol content (mean ± SD)	
			Per fungal dry mass (μg/mg)	Per fungal spore or cell (pg/spore or cell)
Acremonium furcatum	3.0 × 1.6–3.7 × 2.1[a]	8.2	14 ± 7.8	17 ± 1.4
Aspergillus versicolor	2–3.5 (diam)[b,c]	8.0	11 ± 15	1.3 ± 0.2
Aureobasidium pullulans	7.5 × 3.5–16 × 7[b]	24	2.6 ± 16	0.11 ± 0.02
Cladosporium cladosporioides	3 × 2–11 × 5[d]	17	5.6 ± 11	0.33 ± 0.03
Cryptococcus albidus	5 × 3.5–10 × 8[b]	4,300	42 ± 12	$1.1 \times 10^{-4} \pm 0.2 \times 10^{-4}$
Penicillium brevicompactum	3–4.5 (diam)[c,d]	30	5.8 ± 13	0.15 ± 0.03
Rhodotorula minuta	2.3 × 3.5–4.5 × 6.5[e]	2,700	37 ± 6.3	$1.5 \times 10^{-4} \pm 0.2 \times 10^{-4}$
Rhodotorula mucilaginosa	3.2 × 3.9–7.9 × 7.9[e]	840	37 ± 7.6	$6.2 \times 10^{-4} \pm 0.8 \times 10^{-4}$
Stachybotrys chartarum	7 × 4–12 × 10[d]	8.2	12 ± 5.2	4.1 ± 0.21

[a] Data from reference 5.
[b] Data from reference 3.
[c] diam, value is diameter for spherical spores.
[d] Data from reference 15.
[e] Data from reference 7.

broth cultures were incubated at 25°C for 10 (filamentous fungi), 42 (yeasts), or 60 (actinomycete) days. Microbial mass was separated from the broth by filtration (filamentous fungi) or centrifugation at 1,000 × *g* (yeasts and actinomycete). One part of the fungal mass was used for the determination of dry mass content, one was used for counting the total spore numbers, and one was used for ergosterol analysis. Because of the poor growth of *Streptomyces californicus*, the whole yield was used for ergosterol analysis.

Building material samples. Samples of wood chip insulation ($n = 14$), gypsum board ($n = 16$), and glass wood insulation ($n = 10$) were obtained from three buildings with acute or previous moisture damage due to roof leakage, improper drainage, and flaws in design or construction. The samples, one from each sampling site, were taken from constructions with a variety of water damage and microbial contamination. Moisture content, total spore numbers, viable fungal and actinomycete concentrations, and the ergosterol content were determined from subsamples of the materials. Moisture and microbial concentrations were determined immediately after the samples were taken from the buildings. Ergosterol contents were measured within 1 month of the collection of the samples, which were stored at 4°C.

Determination of fungal dry mass and moisture contents of building material samples. Fungal dry mass was determined by drying triplicate samples (0.5 g each) at 105°C for at least 2 h and weighing the samples before and after drying. The dry mass and moisture content of duplicate building material samples were calculated after drying at 105°C for 15 to 20 h. The relative humidities of building materials were calculated from moisture contents by means of the sorption isotherms for each material (VTT Building Technology, Oulu, Finland) (unpublished data).

Microbiological analysis. (i) Viable concentrations. The concentrations of mesophilic fungi (including yeasts), xerophilic fungi (including yeasts), and mesophilic actinomycetes in the building material samples were determined by dilution plating on 2% malt extract agar (Biokar), Dichloran–18% glycerol agar (Lab M, Bury, England), and tryptone-yeast extract-glucose agar (Oxoid Ltd.), respectively, as previously described (14). Dilution plates were incubated at 25°C for 7 (fungi) or 14 (actinomycetes) days. Fungi were identified to the genus level by light microscopy, and results were expressed as CFU per gram of dry mass of material. The detection limits ranged from 40 to 1,430 CFU/g of dry mass. For calculating the mean microbial concentrations, the detection limit divided by 2 was used when no fungi or actinomycetes were isolated from the material sample.

(ii) Total spore numbers. The number of total spores in the fungal broth cultures and in the building material samples were counted with a light microscope by using a Fuchs-Rosendahl counting chamber. For the building material samples, counts were made from the same dilution tubes as those used for viable fungi and actinomycetes. At least 10 areas of the chamber were counted. Total spore concentrations were expressed as spores per milligram of fungal dry mass and spores per gram of dry mass of building material. Fungal and actinomycete spores could not be separated in the analysis.

Ergosterol analysis. Ergosterol contents of the fungal mass and building material samples were analyzed by previously published methods (1, 16). Ergosterol and 7-dehydrocholesterol were purchased from Sigma (St. Louis, Mo.), and *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide was from Fluka Chemie (Buchs, Switzerland). Solvents were of analytical reagent grade, and all glassware was acid washed and heated overnight at 350°C prior to use.

Ergosterol standards (0.5 to 25 μg), fungal mass samples (five replicates of the sample, about 100 mg each), and building material samples (about 1 to 3 g each) were suspended in 3 ml of 10% KOH in methanol, and 0.5 ml of 7-dehydrocholesterol (5.0 μg/mL) was added as an internal standard to each sample. The standards and samples were heated at 80°C for 90 min. Water (1 ml for fungal mass samples, 3 ml for gypsum board samples, and 5 ml for wood chip and glass wool insulation samples) was added, and the aqueous methanol solutions were extracted twice with 2 ml of hexane. The hexane phases were pooled and evaporated to dryness with a nitrogen stream. Samples were dissolved in 1 ml of dichloromethane-hexane (1:1) and applied to a disposable silica gel column which had been preconditioned with 1 ml of diethyl ether and 1 ml of dichloromethane-hexane. The column was washed twice with 1 ml of dichloromethane-hexane and sterols or polar compounds were eluted twice with 1 ml of diethyl ether. After evaporation to dryness with a nitrogen stream, 50 μl of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide and 15 μl of pyridine were added, and the samples were heated at 60°C for 30 min. Trimethylsilyl (TMS)-derivatized samples were dissolved in 500 μl of hexane.

The sterol TMS derivatives were analyzed with a Hewlett-Packard HP 6890 gas chromatograph equipped with an autosampler and a mass selective detector (MSD; Hewlett-Packard 5973). The fused-silica capillary column, HP-5MS (30 m by 0.25 mm [inside diameter] with a film thickness of 0.25 μm), was temperature programmed to increase from 170 to 290°C at a rate of 20°C per 7 min. Both the injector temperature and interface temperature between gas chromatograph and MSD systems were kept at 290°C, and the ion source temperature was 220°C. Injections (2 μl) were made in the splitless mode; helium (1 ml/min) was used as the carrier gas. The MSD was operated in the selected-ion monitoring mode; the ionization energy was 70 eV. The *m*/*z* 337 and 363 ions for the ergosterol TMS derivative and the *m*/*z* 325, 352, and 456 ions for the 7-hydrocholesterol TMS derivative were used in the quantifications. The ergosterol concentrations were expressed as micrograms of ergosterol per milligram of fungal dry mass and micrograms of ergosterol per gram of dry mass of building material. The lowest detectable ergosterol concentration was about 10 pg.

Statistical analysis. Based on graphical analysis and the Kolmogorov-Smirnov test, the distribution of data for the ergosterol content and microbial concentrations in the building material samples was found to be approximately log normal. Therefore, the data were log transformed for computation of Pearson correlation coefficients.

RESULTS

The ergosterol contents per fungal dry mass and nominal spore or cell concentrations of the nine fungal species examined are presented in Table 1. The standard deviation for ergosterol determinations in fungal mass ranged from 5 to 16%, with an average of 12%. This variability was due to the method itself and the heterogeneity of the fungal mass samples. When the ergosterol concentration was calculated on a fungal dry mass basis, the highest ergosterol levels were detected for the yeasts (*Cryptococcus albidus*, *R. minuta*, and *R. mucilaginosa*), and the lowest levels were detected for *Aureobasidium pullulans*, *Cladosporium cladosporioides*, and *P. brevicompactum*. However, the ergosterol content per spore or cell was highest for *Acremonium furcatum*, *Stachybotrys chartarum*, and *Aspergillus versicolor* and lowest for the yeasts. The dry mass content of the fungal cultures varied from 7 to 68%. Dry mass content was lowest in the yeast cultures (7 to 11%) and highest in the cultures of *Acremonium furcatum* and *Aspergillus versicolor* (64 to 68%). The dry mass content of the remaining

fungal cultures ranged from 23 to 32%. No ergosterol could be detected in the actinomycete culture.

The ergosterol levels and microbial concentrations in the building material samples are listed in Table 2. The highest ergosterol levels were detected in the wood chip samples, whereas the highest concentrations of total spores, viable fungi, yeasts, and actinomycetes were found in the glass wool samples. The lowest ergosterol levels and total spore and viable fungal concentrations were detected in the gypsum board samples. The predominant fungi in the building material samples were yeasts and species of *Penicillium*, *Aspergillus*, *Acremonium*, *Cladosporium*, *Scopulariopsis*, and *Exophiala*. In the wood chip samples, mesophilic and xerophilic *Penicillium* species as well as mesophilic and xerophilic *Aspergillus* species comprised on average 48, 49, 8, and 12% of the viable fungi, respectively. The corresponding values were 20, 31, <1, and 6% in the gypsum board samples and 35, 32, 13, and 14% in the glass wool samples. In addition, the mean frequencies of *Acremonium*, *Scopulariopsis*, and *Exophiala* species were 14, 5, and 5% in the wood chip samples and 9, 2, and 1% in the glass wool samples, respectively, whereas xerophilic *Cladosporium* species composed on average 13% of the viable fungi in the gypsum board and 2% in the glass wool samples. Relative humidities of materials were 20 to 100% (mean, 60%; median, 63%) for the wood chip samples, 26 to 30% (mean, 27%; median, 26%) for the gypsum board samples, and 90 to 100% (mean, 98%; median, 100%) for the glass wool samples. The corresponding values for moisture content were 1.8 to 62% (mean, 21%; median, 12%), 0.8 to 2.9% (mean, 1.5%; median, 0.8%), and 0 to 470% (mean, 92%; median, 1.6%), respectively.

Correlation coefficients for ergosterol content and total spore and fungal concentrations in the building material samples are presented in Table 3. For the wood chip samples, an r value of >0.66 and a P value of ≤0.009 were found for the relationship of the ergosterol content and the total spores, the mesophilic and xerophilic fungi, and the mesophilic and xerophilic filamentous fungi. The correlation was higher ($r > 0.75$, $P \leq 0.003$) if the results for the most divergent sample were excluded. In this wood chip sample, the concentrations of total spores, actinomycetes, and especially ergosterol were high, but the fungal concentrations, moisture content, and relative humidity were low. For the gypsum board samples, a correlation between the ergosterol content and viable fungi ($r > 0.48$) was detected, and the correlation coefficients were statistically significant ($P \leq 0.033$), except that for mesophilic fungi. For the glass wool samples, the correlation between ergosterol content and viable fungal concentration was poor. However, the correlation between ergosterol and total spores was quite good, albeit not significant because of the small number of samples. When the results of all the samples were pooled, a significant correlation between the ergosterol content and viable fungal concentration ($r > 0.63$, $P < 0.0001$) was found. The strongest relationship was between ergosterol and filamentous fungi ($r \geq 0.67$, $P < 0.0001$).

DISCUSSION

In the literature, the ergosterol content in fungal cultures is usually expressed in relation to fungal dry mass. Ergosterol concentrations have been reported as 2 to 14 μg/mg for *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* spp., *Cladosporium* spp., and *Penicillium* spp. cultured on Sabouraud dextrose agar for 2 to 4 days (1). Ergosterol contents were reported as 2 to 6 μg/mg for *Alternaria alternata*, *Aspergillus flavus*, and *Aspergillus amstelodami* cultured in liquid media for 2 to 10 days (18) and 0.4 to 1.4

TABLE 2. Concentrations of ergosterol, fungi, yeasts, and actinomycetes in building material samples

Type of building material (no. of samples)	Concn [mean ± SD (median; range)] of:						
	Ergosterol (μg/g)	Total spores (10^7 spore/g)	Mesophilic fungi (10^4 CFU/g)	Mesophilic yeasts (10^4 CFU/g)	Xerophilic fungi (10^4 CFU/g)	Xerophilic yeasts (10^4 CFU/g)	Mesophilic actinomycetes (10^4 CFU/g)
Wood chip insulation (14)	13 ± 16 (9.9; 0.92–68)	9.7 ± 12 (6.3; 0.028–36)	11 ± 18 (3.0; 0.084–68)	0.036 ± 0.059 (≤0.14; f–0.14)	8.6 ± 14 (1.8; 0.021–53)	0.020 ± 0.036 (≤0.14; f–0.11)	5.7 ± 12 (0.36; f–38)
Gypsum board (16)	0.20 ± 0.26 (0.14; 0.083–1.2)	0.99 ± 1.4 (0.65; 0.1–6.0)	0.27 ± 0.51 (0.49; g–1.6)	0.14 ± 0.41 (≤0.011; g–1.6)	0.093 ± 0.21 (0.77; g–0.81)	0.0076 ± 0.017 (≤0.011; g–0.049)	≤0.011 (≤0.011; ≤0.011)
Glass wool insulation (10)	0.89 ± 1.1 (0.34; 0.017–3.5)	39 ± 34 (33.2; 3.4–79)	36 ± 55 (8.2; 0.17–150)	2.4 ± 2.6 (1.7; h–7.1)	20 ± 30 (2.0; 0.078–79)	0.16 ± 0.30 (0.0047; h–0.97)	38 ± 96 (1.9; h–310)

[a] Detection limits: f, 100 to 1,430 CFU/g; g, 40 to 110 CFU/g; h, 690 to 720 CFU/g.

TABLE 3. Correlation coefficients of ergosterol content and fungal concentrations in building material samples

Type of building material (no. of samples)	Correlation [r (P value)] of ergosterol content to concn of[a]:				
	Total spores	Mesophilic fungi	Mesophilic filamentous fungi	Xerophilic fungi	Xerophilic filamentous fungi
Wood chip (14)	0.770 (0.001)*	0.681 (0.007)*	0.674 (0.008)*	0.669 (0.009)*	0.668 (0.009)*
Gypsum board (16)	0.237 (0.377)	0.481 (0.059)	0.535 (0.033)*	0.635 (0.008)*	0.648 (0.007)*
Glass wool (10)	0.428 (0.217)	0.217 (0.546)	0.206 (0.569)	0.186 (0.608)	0.182 (0.615)
All materials (40)	0.418 (0.007)*	0.636 (0.000)*	0.676 (0.000)*	0.665 (0.000)*	0.670 (0.000)*

[a] Asterisks indicate statistically significant correlation coefficients ($P < 0.05$).

μg/mg for *Fusarium culmorum*, *Penicillium rugulosum*, and *Rhizopus stolonifer* cultured on 2% malt extract agar for 3 days (17). In the present study, the average ergosterol content of filamentous fungi varied from 2.6 to 14 μg/mg of dry mass, which agrees with the previously reported values. The higher ergosterol levels per dry mass for the yeasts (average values, 37 to 42 μg/mg) are likely due to the lower dry mass content of the yeast cultures compared to that of the filamentous fungi since vegetative yeast cells have a much lower dry mass per unit weight than fungal spores.

The ergosterol concentrations per nominal spore were 2.5 pg/spore for *Aspergillus versicolor*, 2.6 pg/spore for *P. brevicompactum*, and 3.1 pg/spore for *Cladosporium cladosporioides*, with an average standard deviation of 12%, assuming that the spores are of similar sizes and contain similar amounts of ergosterol (11). In the present study, the average ergosterol values calculated per spore for the fungal species were 1.3, 0.33, and 0.15 pg/spore, respectively. Miller and Young (11) harvested spores from fungal cultures to filters and determined the ergosterol content of the extractions over the range of 5,000 to 25,000 spores. In our study, the ergosterol content was determined for the mycelial cultures, including the content in both spores and hyphae. By calculating the ergosterol content per spore, the hyphal ergosterol content is neglected. Because spore production in fungi is not correlated with hyphal length, estimates of ergosterol concentrations cannot correlate with both hyphae and spore production; therefore, among the filamentous fungal species in this study, the decrease in ergosterol content is due to increasing fungal dry mass. For the same reason, the ergosterol concentrations per spore in this study, in fact, are not comparable to the values reported by Miller and Young (11). The lower ergosterol contents per yeast cell are likely due to the higher cell concentration per dry mass in the yeast cultures in comparison to the spore concentrations in the filamentous fungi.

Ergosterol analysis has recently been suggested for quantitative monitoring of fungi in solid substrates because of the good agreement between hyphal length and ergosterol content and between total ergosterol concentration and mycelial mass, even with more than one fungal species present (13, 17). However, a relationship between ergosterol content and viable fungal spore concentration has not always been observed (2, 17). In some investigations of indoor environments, the ergosterol content of house dust was used as a marker of fungal contamination (1, 10, 16). For example, the ergosterol content in house dust samples ($n = 17$) correlated with the concentration of total viable fungi ($r = 0.65$) and with that of filamentous fungi ($r = 0.57$) in natural-log-transformed data (16), but ergosterol levels and yeast cell concentrations were poorly correlated ($r = 0.25$).

In the present study, the lowest ergosterol contents, total spore concentrations, and fungal levels were found in the gypsum board samples, and the ergosterol content correlated positively with viable fungal concentrations. However, the highest ergosterol levels were found in the wood chip samples, while the viable microbial concentrations were highest in the glass wool samples. This discrepancy may be explained by the higher concentrations of yeasts and actinomycetes with ergosterol absent or present at low levels in the glass wool samples. Therefore, the concentrations of total spores in the glass wool samples were also higher compared with those in the wood chip samples. A positive correlation between ergosterol content and viable fungal concentrations was detected among the wood chip samples, particularly, if the results for one sample with a high ergosterol concentration and low levels of viable fungi and moisture content were omitted. In this sample, the contradictory results might be explained by spore death under drying conditions. Conversion from wet to dry conditions may retard decomposition of ergosterol but it may also increase the loss of viability of fungal spores (11, 13). The reason for very poor correlation coefficients between the ergosterol and viable fungal levels in the glass wool samples remained unclear. However, storage of the samples at 4°C for a few weeks might affect the stability of ergosterol or viable fungal concentrations. Overall, among all the building material samples, a correlation coefficient between the ergosterol content and viable fungal concentrations was similar to that reported earlier for house dust samples (16). Unlike the study of Saraf et al. (16), the present study also indicated a slightly better correlation between ergosterol and filamentous fungi than between ergosterol and total viable fungi including yeasts. This result supports the previous contention that ergosterol is a good indicator of fungal concentrations, particularly for filamentous fungi (12).

Problems of traditional methodology, including poor and variable yields of viable microorganisms, laborious and time-consuming cultural and microscope analyses, and low degree of automation in microbiological investigations, have been well known for a long time. Therefore, the introduction of rapid, cheaper, automated, and more accurate methods is urgently needed for the estimation of biological contamination in indoor environments (4, 11). Ergosterol measurements might be a good alternative to traditional methods. Although the results of the building material samples were logical and promising in the present study, the use of the ergosterol analysis for estimating the stage of fungal contamination in water-damaged building materials needs to be critically evaluated. The degree of variability in the ergosterol content between and within fungal species (e.g., underestimation of yeast concentrations) and the effect of building material type and a history of moisture conditions in the material deserve attention in the future.

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