COMPARISON OF METHODS FOR ASSESSING GROWTH OF FUNGI ON BUILDING MATERIALS

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INTRODUCTION

Microbial growth on building materials and in built environments is a known problem but has drawn more attention in recent years (Ettenauer et al., 2012). Conditions such as floods, water leaks, construction faults, and poor ventilation are among the major causes of moisture accumulation in and on indoor material surfaces (Nevalainen and Seuri 2005). To effectively detect microbial growth on building material surfaces, reliable assay methods for quantifying microbial contamination and/or growth are of utmost importance. The aim of this study was to compare five different methods used in assessing fungal growth on building materials. The methods used in this study were the culture-based method, ergosterol content analysis, N-acetyl hexoseaminidase (NAHA) enzyme activity, quantitative polymerase chain reaction (qPCR) and total spore count.

METHODOLOGIES

Three fungal species, *Cladosporium cladosporioides, Aspergillus versicolor* and *Penicillium brevicompactum,* were inoculated on the inner parts of two acoustic boards and the top surface of wood. Altogether 108 material samples were prepared (3 fungal species x 3 types of materials x 4 time points x 3 repeats). All the building material samples were inoculated by uniformly spreading 0.5 ml of spore suspension (1 x 10^6 spores/ml), prepared in 0.05% Tween 80 onto the material surface using a glass rod. Building material samples inoculated with 0.05% Tween 80 solution without microbial load were used as controls. Inoculated material samples were incubated at room temperature (21°C ± 2) in conditioned chambers (24 L) at a relative humidity of 95 - 97% for 0 days, and 1, 2 and 4 weeks.

Nine plugs cut from the growth surface using sterile arch punch (Elora, Remscheid, Germany) were used for the growth measurements. Three plugs were randomly selected into a sterile tube and extracted with 10 ml of 0.05% Tween 80 for cultivation, total spore counting and qPCR analysis. Another set of three plugs were used for NAHA analysis and the last three plugs for ergosterol analysis.

For cultivation analysis, serial dilutions of the suspensions were prepared and plated in duplicates on 2% Malt extract agar (LabM, Lancashire, UK) for *A. versicolor* and *C. cladosporioides* and on Dichloran glycerol 18% agar (Merck, Darmstadt, Germany) for *P.*

brevicompactum. The fungal concentrations were expressed as colony-forming units per surface area of material (CFU/cm^2). The total concentration of fungal spores ($spores/cm^2$) was determined by a direct counting method with an epifluorescence microscope (Wang et al., 2001). Glass bead DNA extraction (Pitkäranta et al., 2008) and subsequent qPCR assay using Cclad primers for *C. cladosporioides* (Zeng et al., 2006) and PenAsp primers for *A. versicolor* and *P. brevicompactum* (Haugland et al., 2004) was performed. The fungal concentrations were expressed as cell equivalents per cm^2 (cell eq/cm^2).

NAHA enzyme activity was measured from the second set of three building material plugs using the MycoMeter protocol (Reeslev et al., 2003) and expressed as enzyme activity per cm^2 of building material (U/cm^2). The last set of three building material plugs was used for analysis of ergosterol concentration (ng/cm^2) using the method described by Sebastian and Larsson (2003) with minor modifications.

Data were closest to log-normal distribution, and therefore log-transformed data were analyzed calculating the Pearson's correlation and coefficient of variation (CV).

RESULTS AND DISCUSSION

Table 1 shows a summary of concentrations measured with the various assay methods. Comparing the methods that measure CFU/cell/spore counts, it was observed that cultivation gave lowest concentrations and qPCR highest. Culture-base method is inherently affected by the fact that one single CFU often is built from a chain of spores or clumps of conidia, whereas qPCR that measures the DNA content is not affected by spore aggregation. Cultivation-based method measures only culturable whereas total spore count and qPCR method measures both viable and non-viable cells (Lignell et al., 2008). Therefore, the concentrations obtained by qPCR and total spore counting were higher than those obtained by cultivation, as expected. NAHA enzyme activity and ergosterol assays estimate the total biomass utilizing markers specific to fungi. These methods therefore estimate the total fungal content on the material surfaces, and are with this respect are only limited by the stability of the target compound (ergosterol, N-acetyl hexosaminidase) and the method performance.

Table 1. Summary of comparison of the different methods.

	Cultivation (CFU/cm^2)	Total spores (spores/cm^2)	qPCR (cell eq/cm^2)	Ergosterol (ng/cm^2)	NAHA (U/cm^2)
Total number of samples	108	107	100	100	107
Median concentration	14954	62344	184842	186	330
95% confidence interval of median	4705 – 32441	55968 – 101088	91910 – 290191	73 - 611	263 – 498
[1]Coefficient of variation (CV) %	68	23	30	36	26

[1]Average of the CV calculated for each set of three replicate samples

Culture-based method had the highest coefficient of variation indicating a high variation in growth from one replicate agar plate used in the analysis to other. The other methods measured growth directly from the building materials and had smaller variations. Culture-based method may not properly account for total growth. The cultivability in environmental samples has been estimated to vary widely <1 - 79% of the total microbial biomass (Niemeier

et al., 2006). Further, culture-based techniques fail to account for a vast majority of microorganisms which are viable but non-culturable. This implies that the other methods may give more accurate estimates of the total growth with little variations from one replicate of the growth sample to the other.

The Pearson's correlation coefficient, calculated to determine the relationship between the different methods, varied between $0.40 - 0.69$ ($p < 0.01$). The highest correlation was observed between cultivation and qPCR as well as between cultivation and total spore count while the lowest correlation was observed between NAHA and total spore count.

CONCLUSIONS

Although differences between methods for evaluating fungal growth were observed, a moderate to good correlation indicates that the different methods show similar trends. Variation in growth from one replicate material sample to another is expected to result in some variation with all the methods. However, though frequently used, cultivation method was observed to have the highest variation among replicates.

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