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DNA-Based Analyses of Molds in Singapore Public Buildings Results in a Proposed Singapore Environmental Relative Moldiness Index

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Abstract. Dust samples (n=75) were collected from shopping malls, hotels and libraries in Singapore and then analyzed using Mold Specific Quantitative Polymerase Chain Reaction (MSQPCR) for the 36 molds that make up the Environmental Relative Moldiness Index (ERMI). Most of these molds (23/36) occur at similar rates in Singapore and the United States. A Singapore Environmental Relative Moldiness Index (SERMI) is proposed which might be divided into low (<18), medium (18 to 28) and high (>28) mold burden categories but more samples will help to refine these categories.

INTRODUCTION

In 2008, mold and musty smell problems represented 41% of the indoor air quality complaints received by National Environment Agency (NEA) of Singapore. These concerns are warranted based on the Institutes of Medicine's report (IOM, 2004) and World Health Organization's (WHO Europe, 2009) review of the scientific literature which concluded that there was enough evidence to link mold exposures to respiratory illnesses and therefore suggested that exposure to molds should be "minimized". In order to minimize exposures, it is critical that mold assessments are accurate and meaningful.

Most studies of indoor molds have relied on short-term air samples, which were either enumerated by microscopic counting or culturing on various media followed by microscopic identification resulting in data that is not standardized and difficult to interpret (Vesper, 2011). The United States Environmental Protection Agency (US EPA) developed a DNA-based method known as Mold Specific Quantitative PCR (MSQPCR) to identify and quantify 36 mold species, which are common in homes across the US. Of these 36 mold species, 26 species, designated Group 1, occur in water-damaged homes and 10 species, designated Group 2, primarily come from the outside environment. To standardize the interpretation of the results, the Environmental Relative Moldiness Index (ERMI) was developed.

The ERMI is calculated as shown in Eq. 1, by taking the sum of the logs of the concentrations of the 26 Group 1 species (s_1) and subtracting the sum of the logs of the concentrations of 10 Group 2 species (s_2) (Vesper, *et al.*, 2007a).

$$ERMI = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \qquad \text{(Eq. 1)}$$

In this study, the 36 ERMI molds were quantified in dust samples from built environments in Singapore. Based on this data, a Singapore Environmental Relative Moldiness Index (SERMI) is proposed.

MATERIALS AND METHODS

Dust samples (n=75) were collected from 5 shopping malls, 32 libraries and 11 hotels (Figure 2). Floor dust was collected by collecting vacuum bags from the selected location. Dust was retrieved from each vacuum bag and sieved through sterile 300 micron nylon mesh. Then 5.0 +/- 0.1 mg of each dust sample were used for the analysis.

Five mg of dust was added to a Bio101 FASTPREP® tube (Fastprep) containing 0.3 +/- 0.01 g of glass beads (Sigma # G-1277). Each dust sample was spiked with 1 x 10^4 conidia of *Geotrichum candidum* as an external reference, and was then extracted by a rapid mechanical bead-milling method (Haugland, *et al.*, 2004). The extraction tube was shaken in a Fastprep Instrument (Fastprep, Qiagen) for 30 seconds at a setting of 5.5 and the process was repeated 2 more times at 2 minutes interval. The DNA was purified using a DNA-EZ extraction kit (GeneRite, North Brunswick, NJ), according to the manufacturer's instructions.

Analyses were performed on the Light Cycler 480 using the Probes Master reagents (Roche Diagnostics Co, Diethelm, Singapore). All primer and probe sequences, as well as known species comprising the assay groups, were published at the website: http://www.epa.gov/microbes/moldtech.htm.

The difference in each mold species' occurrence rate (% of samples positive/total) in Singapore versus the US was determined using the Fisher Exact Test. A p-value of <0.05 was considered statistically significant. The geometric mean of the concentration of each species (cells/mg dust) for all dust samples was determined using Excel (Microsoft, Seattle, WA, USA), as described previously (Vesper *et al.*, 2007b). The ERMI values from all samples were assembled from lowest to highest and the trend-line equation of the assembled data used to calculate the inflection point and the point where the curve plateaus.

RESULTS AND DISCUSSION

All 36 ERMI molds were found in the built environment dust samples from Singapore, as we had seen previously in a smaller study (Yap, *et al.*, 2009). *Aspergillus penicillioides*, *Aureobasidium pullulans*, *Wallemia sebi*, *Eurotium* group and *Cladosporium sphaerospermum* were the most prevalent species in these indoor Singapore environments (Table 1 and Figure 1). *Cladosporium* spores were the most common fungal spores found in the outdoor air samples from Singapore (Lim *et al.*, 1998). The populations of *Cladosporium* found indoors in our study were likely to be heavily impacted by these outdoor populations. Prakash *et al.* (Prakash, 2005) reported *Aspergillus niger* (92%) as the most prevalent indoor species in carpet dust samples collected from buildings in Singapore, followed by *Curvularia lunata* (51%), *Paecilomycyes variotii* (48%) and *Aspergillus fumigatus* (43%). However, their findings were based on culture using only one type of medium. In addition, air samples and dust samples likely represent different types of mold exposures (Chew, *et al.*, 2003). For example, *A. pullulans* occurs more frequently in dust than air samples (Hyvarinen, *et al.*, 1993; Woodcock, *et al.*, 2006).

Aspergillus penicillioides, *Wallemia sebi* and the *Eurotium* group are often reported from house dust (Samson & van der Lustgraf, 1978) and are known for their xerophilic characteristics (Beguin, 1995). These molds commonly grow on organic materials such as furniture, carpet etc. *A. pullulans* is commonly isolated from dust, wood, textiles etc.

Comparing our overall results with that of US findings, many of the same Group 1 molds that were common in homes in the US were also common in the Singapore indoor built environments (Table 2). This suggests that a Singapore Environmental Relative

Table 1. Geometric mean of the concentrations (number of cells/mg dust) of each species in the dust samples from each indoor location, i.e. Mall (n=16), Library (n=32) and Hotel (n=11)

Group 1	**Mall**	**Library**	**Hotel**
Aspergillus flavus	15.8	2.8	3.1
Aspergillus fumigatus	1.5	0.9	1.0
Aspergillus niger	10.7	7.8	1.7
Aspergillus ochraceus	ND	92.5	ND
Aspergillus penicillioides	13310.8	20220.0	4163.4
Aspergillus restrictus	0.3	0.3	0.5
Aspergillus sclerotiorum	1.0	1.4	3.1
Aspergillus sydowii	57.8	21.2	24.4
Aspergillus unquis	15.5	2.8	13.2
Aspergillus versicolor	36.4	19.5	14.9
Aureobasidium pullulans	4079.4	5688.6	2146.8
Chaetomium globosum	49.4	28.7	23.5
Cladosporium sphaerospermum	42.4	9.7	4.2
Eurotium group	18.2	12.3	6.2
Paecilomyces variotii	7.5	2.8	2.2
Penicillium brevicompactum	5.0	1.6	2.3
Penicillium corylophilum	ND	13.9	74.9
Penicillium group 2	ND	121.8	ND
Penicillium purpurogenum	1.5	0.7	0.7
Penicillium spinulosum	146.5	72.8	105.2
Penicillium variabile	9.7	4.2	3.3
Scopulariopsis brevicaulis	0.9	0.5	0.4
Scopulariopsis chartarum	4.0	2.8	3.1
Stachybotrys chartarum	0.4	0.2	0.4
Trichoderma viride	27.9	12.7	25.2
Wallemia sebi	97.3	190.9	99.5
Group 2			
Acremonium strictum	35.2	8.6	ND
Alternaria alternata	2.1	0.3	1.2
Aspergillus ustus	0.6	1.0	1.2
Cladosporium cladosporioides type 1	9.2	14.8	9.5
Cladosporium cladosporioides type 2	5.8	0.3	0.8
Cladosporium herbarum	6.3	1.9	8.4
Epicoccum nigrum	18.3	23.5	61.7
Mucor group	2.8	3.1	1.4
Penicillium chrysogenum type 2	2.1	2.1	3.3
Rhizopus stolonifer	0.5	0.3	0.3

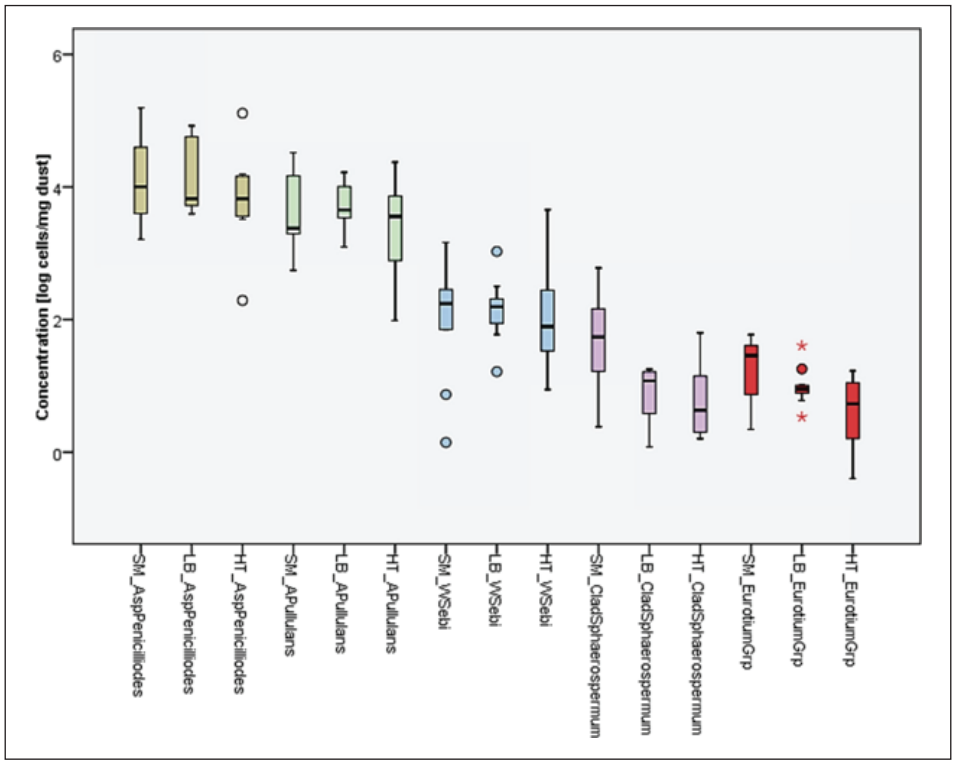

Figure 1. Box plots of the concentrations of the 5 most prevalent mold species in Singapore indoor environments. SM stands for Shopping Mall, LB for Library and HT for Hotel. Circles represents outliers with values that differ from the median by 1.5 to 3 times the interquartile range (IQR, represented by the height of the box) while asterisks represents extreme outliers with values that differ from the median by more than 3 times the IQR.

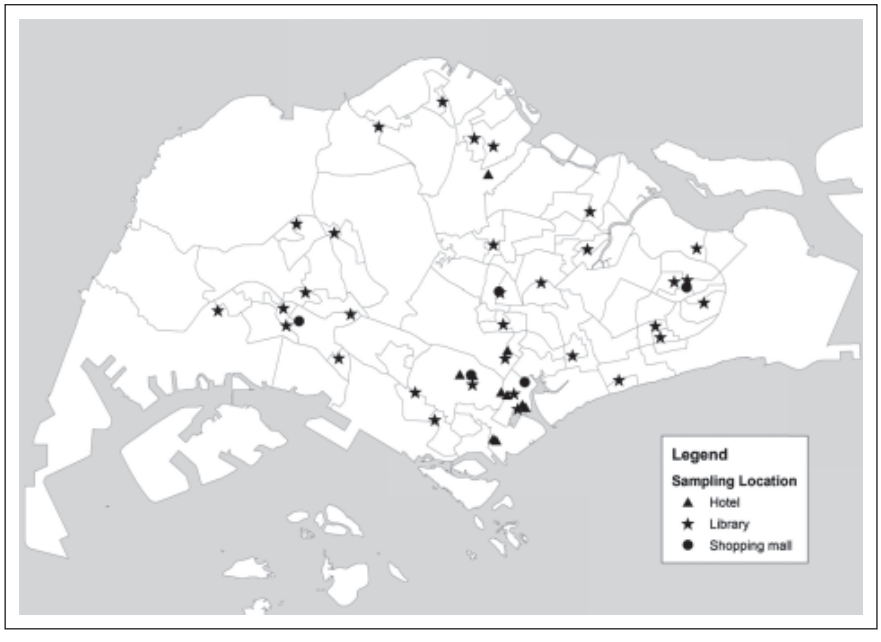

Figure 2. Map of Singapore showing the locations of the shopping malls, libraries and hotels where samples for the study were collected from.

Table 2. Occurrence rate (as percentage % positive /total number of samples) of each species in dust samples from Singapore compared to the US (Vesper *et al.*, 2007a). Those significantly different (*) are bolded.

	% Singapore	% US	Fisher's Exact test P <0.05
Group 1			
Aspergillus flavus	63	36	
Aspergillus fumigatus	25	62	
Aspergillus niger	81	69	
Aspergillus ochraceus	**7**	**27**	*
Aspergillus penicillioides	100	90	
Aspergillus restrictus	4	12	
Aspergillus sclerotiorum	25	26	
Aspergillus sydowii	***85***	***29***	*
Aspergillus unquis	***59***	***20***	*
Aspergillus versicolor	80	30	
Aureobasidium pullulans	100	94	
Chaetomium globosum	95	51	
Cladosporium sphaerospermum	95	82	
Eurotium group	96	98	
Paecilomyces variotii	72	46	
Penicillium brevicompactum	**13**	**52**	*
Penicillium corylophilum	8	17	
Penicillium group 2	**1**	**8**	*
Penicillium purpurogenum	23	15	
Penicillium spinulosum	**61**	**20**	*
Penicillium variabile	60	50	
Scopulariopsis brevicaulis	**15**	**53**	*
Scopulariopsis chartarum	71	38	
Stachybotrys chartarum	**2**	**38**	*
Trichoderma viride	59	27	
Wallemia sebi	97	75	
Group 2			
Acremonium strictum	**3**	**57**	*
Alternaria alternata	**19**	**88**	*
Aspergillus ustus	19	40	
Cladosporium cladosporioides type 1	95	99	
Cladosporium cladosporioides type 2	**20**	**70**	*
Cladosporium herbarum	71	74	
Epicoccum nigrum	64	93	
Mucor group	75	92	
Penicillium chrysogenum type 2	**21**	**66**	*
Rhizopus stolonifer	**3**	**29**	*

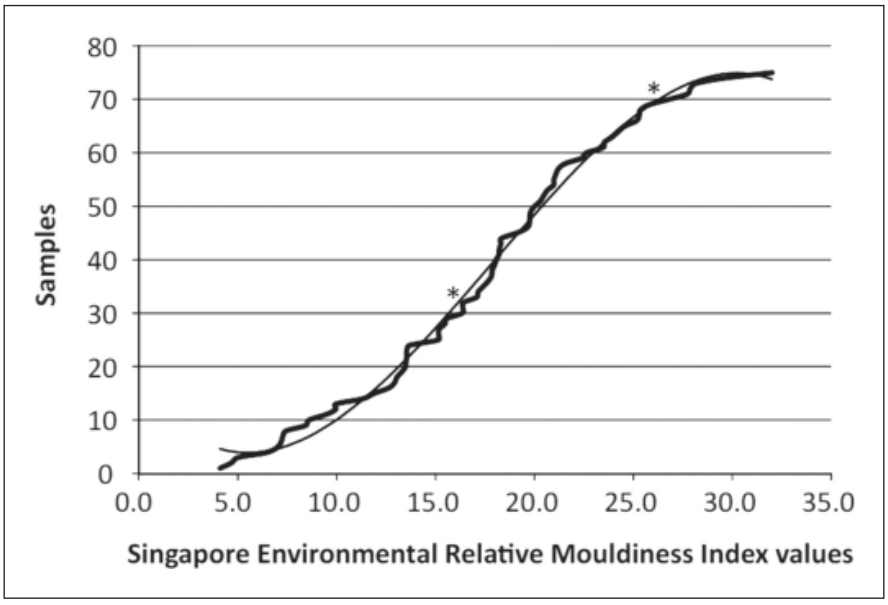

Figure 3. The Singapore ERMI value for each sample was calculated then assembled from lowest to highest. The trend-line (narrow line) fits the equation $y = -0.0094x^3 + 0.5035x^2 - 4.695x + 16.061$. The inflection point, based on the second derivative, is at approximately 18 and the plateau change point is at approximately 28 (*).

Moldiness Index (SERMI) can be proposed for indoor environments based on the same species composition that was used in the US.

Using equation 1, the ERMI values were calculated for each of the samples from all environments sampled in Singapore and assembled from lowest to highest (Figure 3). This plot shows a typical accumulated distribution curve similar to what was found for US homes (Vesper *et al.*, 2007a). The fitted trend-line is a third order equation which has an r value equal to 0.9935. There are two points at which the slope of the curve changes, 18 and 28. The second derivative is 0 when x=17.76 (approximately 18). This means that the rate of change of the gradient of the fitted curve is positive when x<18 and negative when x>18. In addition, the trajectory of the curve changes again at a value of approximately 28, where it begins to plateau. We propose the SERMI might be divided into low (<18), medium (18 to 28) and high (>28) mold burden categories.

MSQPCR is a standardized, rapid (results in about 2 hours) tool for mold identification and quantification. Knowing the prevalence and abundance of the species and the ERMI value was shown to help discover hidden mold problems (Vesper *et al.*, 2009). A possible SERMI for Singapore is proposed. However, a more comprehensive, random national survey will need to be conducted to refine the SERMI for employment broadly in indoor air quality investigations in Singapore.

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NOTICE

The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development, collaborated in the research described here. It has been subjected to the Agency's peer review and has been approved

as an EPA publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use. Commercial use of the ERMI technology can provide royalties to the EPA.

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