Assessment of mold concentrations in Singapore shopping centers using mold-specific quantitative PCR (MSQPCR) analysis

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Abstract Molds can pose a human health threat and may amplify in buildings in humid climates. The objective of this study was to evaluate the mold growth in Singapore shopping centers based on the collection of 40 dust samples from 15 shopping centers, including one with a history of water damage. The dust was analyzed by a DNA-based technology called mold-specific quantitative PCR (MSQPCR). In a water-damaged shopping center, most of the 26 water-damage indicator species were detected at some concentration and many were much more abundant than the average in the shopping centers. MSQPCR is a useful method for quantifying indoor molds in tropical climates.

Keywords Mold · Shopping centers · Singapore · MSQPCR

Introduction

The presence of excessive mold growth can contribute to respiratory illness [1, 2] and possibly infections [3]. In addition to water leaks in a building, a humid climate may promote mold growth indoors. Indoor dampness and mold in children's bedrooms were important risk factors associated with allergic symptoms in young children in Singapore [4]. Also, mold sensitization was significantly more common in children whose Singapore homes lacked air conditioning [5]. The objective of this study was to assess the mold growth in Singapore shopping centers using a DNA-based method of mold analysis called mold-specific quantitative PCR (MSQPCR) [6].

A total of 15 shopping centers, widely distributed across Singapore, were selected for this study. For each center, the entrance level, a mid-level and food-court level (usually the top level) were investigated for visible mold and the air-filter in the air handling unit serving each level was recovered, representing about one month of dust accumulation. Each air filter was processed to recover the dust by washing in water. The resulting dust suspension was centrifuged at 4,000 × g for 10 min and the dust pellet left to dry at 65°C. The dried dust pellet was then sieved through sterile 300 micron nylon mesh and 5 mg extracted by a rapid mechanical bead-milling method [6] and DNA purified with the use of DNA-EZ extraction kit (GeneRite, North Brunswick, NJ). MSQPCR 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>.

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Table 1 Spores per mg dust for the analysis of the 40 samples, from 15 shopping centers, for each of the 26 Group 1 and 10 Group 2 molds

Mold species/Groups			
Group 1	GM	AVG	Highest
<i>Aspergillus penicillioides</i>	4879	28868	290899
<i>Aureobasidium pullulans</i>	564	93509	3657236
<i>Wallemia sebi</i>	92	1094	18132
<i>Penicillium spinulosum</i>	42	595	14844
<i>Aspergillus sydowii</i>	28	118	587
<i>Eurotium</i> group	23	328	6105
<i>Aspergillus niger</i>	13	17216	643078
<i>Paecilomyces variotii</i>	11	40	229
<i>Aspergillus versicolor</i>	8	38	310
<i>Cladosporium sphaerospermum</i>	7	632	24252
<i>Chaetomium globosum</i>	7	49	430
<i>Penicillium variabile</i>	6	25	158
<i>Aspergillus flavus</i>	5	22	342
<i>Aspergillus unguis</i>	5	16	181
<i>Aspergillus ochraceus</i>	3	21	146
<i>Trichoderma viride</i>	3	11	95
<i>Penicillium brevicompactum</i>	3	22	349
<i>Penicillium purpurogenum</i>	3	31	626
<i>Scopulariopsis chartarum</i>	2	6	37
<i>Aspergillus fumigatus</i>	2	3	17
<i>Penicillium corylophilum</i>	2	10	164
<i>Penicillium</i> group 2	2	13	203
<i>Scopulariopsis brevicaulis</i>	2	3183	127257
<i>Aspergillus restrictus</i>	1	2	14
<i>Aspergillus sclerotiorum</i>	1	1	8
<i>Stachybotrys chartarum</i>	1	1	2
Group 2			
<i>Cladosporium cladosporioides</i> 1	9	64	1393
<i>Penicillium chrysogenum</i> 2	2	5	47
<i>Mucor</i> and <i>Rhizopus</i> group	2	4	21
<i>Epioccum nigrum</i>	2	8	110
<i>Aspergillus ustus</i>	2	12	402
<i>Cladosporium herbarum</i>	1	2	18
<i>Rhizopus stolonifer</i>	1	3	37
<i>Cladosporium cladosporioides</i> 2	1	3	77
<i>Alternaria alternata</i>	1	1	2
<i>Acremonium strictum</i>	0	0	0

The molds are arranged from highest geometric mean (GM) to lowest, with the associated average (AVG). Also the highest concentration in any of the samples tested is also given.

Methods for estimating the amplification factors and extrapolating concentrations from the standard curves have been described previously [7, 8].

Singapore shopping centers are public buildings and therefore the NEA is concerned about the exposures in those environments. In this study, 36 indicator species were

enumerated using MSQPCR. These 36 indicator molds make-up the Environmental Relative Moldiness Index (ERMI) that was created to describe the mold burden in US homes [9]. Higher ERMI values are associated with increased risk of asthma in children [10]. The 26 species

called Group 1 were found to be indicators of water-damage and mold growth in the US.

Many of the Group 1 molds were measured in Singapore shopping centers (Table 1) at higher concentrations than found in the US and UK homes [9, 11]. The high temperatures

Table 2 Concentration of spores (number per mg dust) in dust samples from the top, middle and bottom levels of a water-damaged shopping center

Mold species/groups	Top	Middle	Bottom
Group 1			
<i>Aspergillus penicillioides</i>	50842	290899	30077
<i>Aureobasidium pullulans</i>	936	1100	266
<i>Wallemia sebi</i>	10994	18133	2857
<i>Penicillium spinulosum</i>	14844	1368	132
<i>Aspergillus sydowii</i>	430	361	39
<i>Eurotium</i> group	4290	6106	465
<i>Aspergillus niger</i>	30197	13227	1186
<i>Paecilomyces variotii</i>	52	203	5
<i>Aspergillus versicolor</i>	183	82	9
<i>Cladosporium sphaerospermum</i>	0	2	0
<i>Chaetomium globosum</i>	245	11	0
<i>Penicillium variabile</i>	155	102	5
<i>Aspergillus flavus</i>	29	9	3
<i>Aspergillus unguis</i>	16	181	0
<i>Aspergillus ochraceus</i>	146	26	0
<i>Trichoderma viride</i>	30	11	0
<i>Penicillium brevicompactum</i>	349	154	167
<i>Penicillium purpurogenum</i>	366	626	126
<i>Scopulariopsis chartarum</i>	26	0	0
<i>Aspergillus fumigatus</i>	17	16	17
<i>Penicillium corylophilum</i>	91	164	0
<i>Penicillium</i> group 2	56	0	0
<i>Scopulariopsis brevicaulis</i>	0	0	0
<i>Aspergillus restrictus</i>	12	14	0
<i>Aspergillus sclerotiorum</i>	3	0	0
<i>Stachybotrys chartarum</i>	0	0	0
Group 2			
<i>Cladosporium cladosporioides</i> 1	135	12	4
<i>Penicillium chrysogenum</i> 2	47	11	0
<i>Mucor</i> and <i>Rhizopus</i> group	15	13	2
<i>Epioccum nigrum</i>	0	0	0
<i>Aspergillus ustus</i>	2	402	0
<i>Cladosporium herbarum</i>	0	0	0
<i>Rhizopus stolonifer</i>	37	30	5
<i>Cladosporium cladosporioides</i> 2	6	0	0
<i>Alternaria alternata</i>	0	0	0
<i>Acremonium strictum</i>	0	0	0

and humidity levels, especially at night if the air-conditioning in the shopping centers is switched off, might contribute to the growth of Group 1 molds. Of course, water intrusion can enhance the amplification of the Group 1 molds.

The mold concentrations in the three samples from the shopping center with a known history of water problems are shown in Table 2. In this water-damaged shopping center, many of the Group 1 species were found at very high concentrations and most of the 26 Group 1 species were detected at some concentration (Table 2). Many Group 1 species were more common than the average values found in all centers combined (Table 1, 2nd data column). The concentrations of Group 1 molds from the top level filter were the greatest (Table 2), followed by middle level and then entrance level. This suggests that one possible source of the moisture might have been a leaky roof.

A recent review of bio-aerosols in Indian buildings described the “urgent need to undertake the study of indoor air, to generate baseline data and explore the link to nosocomial infections” [12]. Monitoring these environments with an objective analytical tool like MSQPCR may be useful in those studies.

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