(1→3)-ß-D-GLUCAN IN SOME INDOOR AIR FUNGI

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Abstract

The amount of (1→3)-ß-D-glucan was measured in three species of fungi during different phases of growth and after heatkilling. The amounts were the same in living and dead spores, but not related to the growing time intervals or media studied. The amount of (1→3)-ß-D-glucan was significantly higher in *Stachybotrus atra* than in *Penicillium aurantiogriseum* and *Aspergillus fumigatus*. The results suggest that airborne (1→3)-ß-D-glucan may be a better indicator of risk than airborne viable or dead spores.

Background

Moulds in indoor air have been related to symptoms of respiratory disease and general symptoms of tiredness and headache in numerous studies [eg 1, 2, 3, 4]. The most common method to determine the presence of moulds in indoor air is to measure the number of viable organisms although the results are difficult to use for quantitative risk assessments [5, 6, 7].

The symptom profile reported from the different studies suggests that the underlying reason for the symptoms in the majority of cases is an airways inflammation [8]. Allergic responses are only seldom encountered. Moulds contain (1→3)-β-D-glucan which are polyglucose compounds present in the cell wall of fungi and some bacteria. They have a variety of effects on the inflammatory and immune systems and act synergistically with endotoxin to produce inflammation [9, 10].

Some studies suggest a relation between the presence of (1→3)-β-D-glucan in indoor air and the extent of airways inflammation [11, 12] and some symptoms of inflammation have been produced by exposure to pure (1→3)-β-D-glucan [13]. In view of the possibility that determinations of (1→3)-β-D-glucan could be a better predictor for the risk than determinations of viable organisms, a knowledge about the relationship between viable moulds, detected with traditional techniques and the amount of (1→3)-β-D-glucan is of interest.

To evaluate this relationship in an experimental setup, we investigated three common indoor fungi and determined the relationship between the number of viable and dead spores and the amount of (1→3)-β-D-glucan.

Material and methods

Fungi

The species *Penicillium aurantiogriseum*, *Aspergillus fumigatus* and *Stachybotrus atra* were investigated. The fungi were grown on rice - a nutritious granulated media - or wood chips, the latter to simulate a building material.

Parboiled rice was rinsed under lukewarm water until the rinse water was clear. Moist rice (100 g) was weighed into 1 liter flasks and autoclaved at 120°C for 20 min. The flasks were inoculated with 6 ml of a spore suspension of the organism to be studied (10^7/ml sterile water) and incubated at 30°C. After 5-7 days the culture was dried in room air.

Pine wood chips (2x3x0.5 mm) were wetted with sterile water (100 g wood chips and 100 g water) for one hour. Excess water was poured off and the wood chips were inoculated with 6 ml of the spore suspension, incubated at 30°C for 10-14 days and then air dried.

Aerosolization of spores

The cultures of rice or wood chips were placed in a horizontal drum (length 70 cm, diameter 40 cm) rotating at 10 r.p.m. to generate an aerosol of spores. Samples of the aerosol were taken from the interior of the rotating drum by drawing air through filters (Millipore AAWP, pore size 0.8 µm) at a flow rate of about 5 l/hour.

The filters were shaken in water and a sample was placed in a Bürker chamber and the number of spores counted. Practically 100% of the particles in the aerosols were spores.

Analysis of (1→3)-β-D-glucan

The filters were shaken for 10 minutes in pyrogen free water and this was analyzed for (1→3)-β-D-glucan using specific Limulus lysate [14]. Filter extract samples of 50 µl were placed in a microwell plate and 50 µl glucan specific lysate (Fungtox®, Seikagaku, Tokyo, Japan) were added. The plate was incubated in a spectrophotometer (Wellreader®, Seikagaku Co, Tokyo, Japan) and the kinetics of the ensuing color reaction was read photometrically and transformed into absorbance units at the maximum slope of the curve. The absorption was compared to a standard (1→3)-ß-D-glucan (Pachyman, Seikagaku, Tokyo, Japan) and the result expressed as ng/ml liquid. Using the value for air flow through the filter, this value was transformed to ng/m^3. The detection limit for this technique is 10 picogram/ml, which corresponds to 0.7 ng/m^3 during the sampling circumstances prevailing in this study.

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Results

Table 1 shows the amount of (1→3)-β-D-glucan per 10^7 spores on filters from the different fungi in ranking order. It is seen that the major groups of samples from Penicillium and Aspergillus contained up to 10 ng/10^7 spores. Occasional samples contained more than 50 for these two species. Regarding levels in Stachybotrus spores, there were no samples containing less than 50 ng/10^7 spores. Values for dead and alive spores were very similar.

Figure 1 compares the median value for (1→3)-β-D-glucan from the three species tested.

It is seen that the amounts of (1→3)-β-D-glucan in viable or dead *Stachybotrus* spores were significantly higher than in the other two species. For *Penicillium*, there was a tendency for dead spores to contain slightly more (1→3)-β-D-glucan although the difference was not statistically significant.

Examinations at 1, 2, and 3 weeks after inoculation showed that the amount of (1→3)-β-D-glucan/spore was not significantly different between the different time periods studied (data not shown). Neither was there a difference between growth on rice or wood chips.

Comments

The techniques used in the study to cultivate fungi and to generate aerosols are straight forward and have been used in previous experiments [15,10]. To determine (1→3)-β-D-glucan in the minute amounts present here, it is necessary to use a biological reagent. This has only recently been introduced commercially and the general experience with this technique is thus limited. On the other hand, it has been used over a number of years in experimental studies and relationships have been found between effects and the amounts of (1→3)-β-D-glucan in the environment [11-13].

In this experiment, the values were referred to the number of spores, which obviously increases the potential methodological errors. In spite of these shortcomings, some conclusive results emerged from the study.

We could not demonstrate a difference in the amount of (1→3)-β-D-glucan in dead or alive spores. This is reasonable, as (1→3)-β-D-glucan is a resistant substance and very resistant to chemical and physical agents. From a practical point of view, this means that determinations of viable molds may severely underestimate the biologically relevant dose present in the studied environment.

The results also clearly demonstrated that there was a difference in the amount of (1→3)-β-D-glucan between different mould species. *Stachybotrus* had the highest values and this spore is about three times larger than the spores from the other species studied. The amount of (1→3)-β-D-glucan per unit weight of spore might thus be the same but from a practical point of view, a risk estimation based on the number of spores may be erroneous.

This study has not addressed the question whether (1→3)-β-D-glucan from *Stachybotrus* is more toxic on a per weight basis, compared to (1→3)-β-D-glucan from the other species studied. In view of the severe effects related to *Stachybotrus* exposure in terms if infants' pneumonia, such studies should be performed [16, 17].

We could not detect any difference between sampling at different times after inoculation or growth on rice or wood chips. This suggests that formation of (1→3)-β-D-glucan within the spore is a robust process that is little influenced by environmental conditions.

In summary, the results from this investigation suggest that determinations of the amount of (1→3)-β-D-glucan in mold infested interiors, may provide a more precise appraisal of the risks involved.

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Table 1. Amount of (1→3)-ß-D-glucan in spores of Aspergillus, Penicillium and Stachybotrus. Samples of aerosolized viable and dead spores in ranking order (ng/10^7 spores).

Penicillium		**Aspergillus**		**Stachybotrus**	
alive	dead	alive	dead	alive	dead
0.5	11.6	0.8	00.2	86.3	1134
2.1	42.5	0.9	0.5	123	693
3.6	45.7	1	3.7	257	1.8
5.2		2.1	125	264	
5.2		2.3	207	391	
7.1		2.4		402	
7.7		2.7		967	
10.1		3.1		5390	
19.8		4		3933	
20.2		11			
38		26			
84		28			
117		28			
178		31			
		36.5			
		42			
		45			
		45			
		65.3			

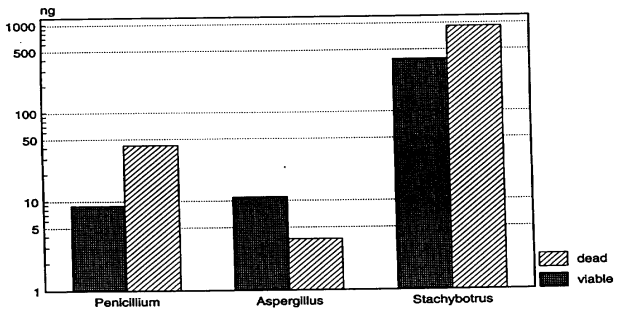

Figure 1. Amount of (1→3)-ß-D-glucan in spores of viable and dead Penicillium, Aspergillus and Stachybotrus (ng/10^7 spores). Median values.