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The relation between growth of four microbes on six different plasterboards and biological activity of spores

Abstract Microbial growth on water-damaged building materials is commonly associated with adverse health effects in the occupants. We examined the growth of *Stachybotrys chartarum*, *Aspergillus versicolor*, *Penicillium spinulosum*, and *Streptomyces californicus*, isolated from water-damaged buildings, on six different brands of plasterboards. The microbial growth was compared with the biological activity of the spores, that is the potential to induce cytotoxicity and proinflammatory mediators in RAW264.7 macrophages. These results showed that the microbial growth on plasterboard depended on both the microbial strain and the brand of plasterboard used. The biological activity of spores appeared to be regulated by different growth conditions on plasterboards so that good microbial growth was associated with a low bioactivity of the spores, whereas the spores collected from plasterboard supporting only weak growth usually were biologically active. Cytotoxicity of either *S. chartarum* or *A. versicolor* did not correlate with any particular growth conditions or induced inflammatory responses. Instead, there were positive correlations between cytotoxicity and levels of induced proinflammatory cytokines for *P. spinulosum* and *S. californicus*. These data suggest that both the microbial growth on plasterboard and the resulting bioactivity of spores vary and might be affected by changing the growth conditions provided by the plasterboards.

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Key words: Microbial growth; Water-damaged building; Macrophages; Cytotoxicity; Cytokines.

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Practical Implications
Microbial growth on water-damaged building materials may lead to production of biologically active compounds capable of inducing inflammatory and toxic reactions in mammalian cells. Growth conditions provided by the wetted material affect the ability of microbial spores to induce these immunological reactions. This should be taken into account when developing microbiologically safer building materials.

Introduction

Microbial growth on building materials as a result of moisture is commonly associated with adverse health effects, such as respiratory symptoms, infections and increased risk for asthma and allergy (Koskinen et al., 1995, 1999; Hodgson et al., 1998). However, the mechanisms leading to these events remain unclear. We have demonstrated that the spores of *Stachybotrys chartarum* (*atra*), *Aspergillus versicolor*, *Penicillium spinulosum*, and streptomycetes, isolated from water-damaged buildings, can cause cell mortality, as well as inducing a dose-dependent production of important inflammatory mediators, such as nitric oxide (NO), tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) in cell cultures (Hirvonen et al., 1997a,b; Ruotsalainen et al., 1998; Jussila et al., 1999; Murtoniemi et al., 2001). In the presence of excess moisture and readily available nutrients, microbial growth occurs, especially in aged organic building materials containing cellulose, e.g. wooden materials, jute, wallpaper, and cardboard (Gravesen et al., 1999). Plasterboards are widely used building materials. However, the boards can vary in detailed composition and material content. Typically, starch and sugars are bound into the core, and a slightly different liner may be used on the front side of the plasterboard compared with the rear side.

Nutritional conditions like nitrogen or phosphorus starvation are generally known to induce a cascade of metabolic changes permitting the adaptation to stress conditions and giving competitive advantage against

other microorganisms. Under these conditions organisms enter a period of slower growth and undergo morphological alterations and changes in metabolism known as secondary metabolism. The majority of bioactive products of microorganisms, including antibiotics, mycotoxins, pigments, and enzyme-inhibitors are generated during secondary metabolism (von Döhren and Gräfe, 1997). Species of *Stachybotrys*, *Aspergillus* and *Streptomyces* infesting in building materials have been reported to produce toxins such as trichothecenes, sterigmatocystin and valinomycin, respectively (Nikulin et al., 1994; Andersson et al., 1998; Nielsen et al., 1998a,b). Because of a huge range of bioactive compounds originating from microorganisms, it is impossible to determine all of the harmful microbial metabolites produced on building materials. Instead, we hypothesized that the spore-induced cytotoxicity and production of inflammatory mediators in macrophages could be used as an index of the biological activity of microbial spores synthesized on different growth conditions.

We examined the growth of *S. chartarum*, *A. versicolor*, *P. spinulosum*, and *Streptomyces californicus* on six different brands of plasterboards. We also evaluated the relation between these growth results and earlier determinated biological activities of the spores, assessed as their potential to evoke cytotoxicity and induce the production of TNF-α, IL-1β, NO, and IL-6 in RAW 264.7 macrophages (Murtoniemi et al., 2001).

Materials and methods

Isolation and identification of microbial strains

Three fungal strains, *S. chartarum* HT580, *A. versicolor* HT486 and *P. spinulosum* HT581, and a gram-positive bacterium, *Streptomyces californicus* VTT E-99–1326 (A4) (VTT, Technical Research Center of Finland, Biotechnology and Food Research), were used in this study. *Aspergillus versicolor*, *P. spinulosum*, and *S. californicus* were isolated from indoor air of mould problem buildings by the six-stage impactor (Graseby Andersen, Atlanta, GA, USA). *Stachybotrys chartarum* was isolated from a material sample of a mould-damaged building. The fungal strains were identified morphologically at the Centraalbureau voor Schimmelcultures (Baarn, the Netherlands). *Streptomyces californicus* was identified on the basis of partial 16S rDNA sequence and physiological tests at the German Collection of Microorganisms and Cell Cultures (DSMZ).

Growth conditions

Stachybotrys chartarum, *A. versicolor*, and *P. spinulosum* were cultivated on 2% malt-extract agar (MEA) at 25°C, and *S. californicus* on tryptone – yeast extract – glucose (TYG) agar (Bacto Plate Count Agar, Difco, Detroit, MI, USA) at 20–23°C in the dark until they sporulated. The spores of each strain were collected with a sterile plastic rod to 10 ml of Hanks balanced salt solution (HBSS) (Gibco Laboratory, Paisley, UK). Six different brands of unused plasterboards that are commercially available in several European countries and some of them also in USA were used in this study. Pieces of six different plasterboards, 171 cm^2 in area, were oven-sterilized (170°C, 1 h), and then wetted with 77 ml of sterilized water. The amount of 10^6 spores in 0.5 ml was inoculated at the area of 81 cm^2 on the front side of one board, and on the rear side of the other board of the same brand. Two pieces of the same plasterboard, inoculated on opposite sides, were placed in a tight, sterilized 4 l-glass vessel containing a thin layer of sterilized water at the bottom. To avoid direct contact with water, the pieces were placed vertically on the top cover of a Petri dish lying above the water surface. Identically treated plasterboards without inoculations were used as controls. The vessels were kept at the room temperature of 20–23°C, and aerated with filter-sterilized (0.2 μm, Schleicher & Schuell GmbH, Germany) air once a day for 10 min at a flow rate of 400 ml/min.

Growth analyzes

The microbial growth and sporulation were observed visually three times a week until no changes occurred during the next 14 days. The growth was evaluated as the percentage (20, 40, 60, 80 and 100%) of the maximum amount of biomass achieved for each microbe. From the obtained growth curve the growth rate was determined as the slope in the exponential growth phase. At the end of the experiment, the spores were collected from the inoculated area with sterilized swab, and transferred to 5 ml of HBSS buffer. The spore concentration of *S. chartarum*, *A. versicolor*, and *P. spinulosum* suspensions was counted using light microscopy and Bürker counting chamber. The spore density of *S. californicus* was determined by counting the spores after the acridine orange staining using an epifluorescence microscopy (Palmgren et al., 1986). The liner and core materials were examined with the light microscopy to define extent of growth.

Biological activity analyzes

The mouse macrophage cell line RAW264.7 (American Type Tissue Collection; Rockville, MD, USA) was grown at 37°C, 5% CO_2, in RPMI Medium 1640 (Gibco Laboratory, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (HyClone Laboratory, Logan, UT, USA), 1% L-glutamine, and 1% PNS antibiotic mixture (Gibco Laboratory, Grand Island, NY, USA). The cells were then exposed to the

spores of *S. chartarum*, *A. versicolor*, *P. spinulosum* and *S. californicus*, collected from different plasterboards, at the doses of 10^4, 10^5 and 10^6 spores/10^6 cells for 24 h.

Nitric oxide was assayed in the culture medium by Griess method. Cytokines were analyzed using enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, MN, USA). Cytotoxicity of the spores in the cell culture was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,3-dimethyltetrazolium bromide (MTT) test. All these assays and detailed results are presented in Murtoniemi et al. (2001).

Statistical analyzes

Growth, inflammatory response and cytotoxicity data were subjected to principal component analysis (PCA) to elucidate major variation and covariation patterns for each microorganism using SPSS for Windows version 9.0 (SPSS, Inc., Chicago, IL, USA). In PCA the original data matrix is transformed into a new set of fewer uncorrelated variables called principal components. The PCA results are presented as biplots of loadings of each variable, and score plots of sample points. The higher the loading of a variable, the more influence it has in the formation of the principal component score and vice versa (Wold et al., 1987). The ability of the microbial spores to induce production of inflammatory mediators and to cause cytotoxicity was tested at a dose of 10^6 spores/10^6 cells in the PCA. IL-6 and TNFα production induced by spores of *S. californicus* was tested at a dose of 10^5 spores/10^6 cells because the maximum effect was achieved with this dose. The correlations between growth parameters, inflammatory responses and cytotoxicity of four microbes were studied by the Pearson correlation analysis.

Results

Microbial growth on plasterboards

The microbial growth, evaluated as the duration of lag phase, growth rate, and the amount of mycelium and spores synthesized, varied greatly between the different brands of plasterboards, and was dependent on the microorganism used. *Stachybotrys chartarum* and *A. versicolor* started to grow on all plasterboards within 5–9 days from inoculation (Table 1), whereas the growth of *P. spinulosum* started 10–23 days and *S. californicus* as late as 20–34 days after inoculation. One exception to these results was the growth of *A. versicolor* on plasterboard 6, which started only after 20–23 days. The growth rates of *P. spinulosum*, *A. versicolor*, and *S. chartarum* on different brands of plasterboards varied considerably, although that of *S. californicus* was fairly constant. The amount of mycelium produced varied between 40 and 100% of the maximum for *S. chartarum* and *P. spinulosum*, and between 60 and 100% for *A. versicolor* and *S. californicus*. However, it is important to note that the visual evaluation of the amount of mycelium and spores produced without biomass determination enabled the grading within one strain, but did not allow the comparison between different microbes with different morphologies. The growth of all microbes on all plasterboards studied occurred only on the liners, not in the core.

Growth and biological activity of spores

After growth, the spores of four microbes collected from different plasterboards were applied to the RAW264.7 macrophages. For each microbe, growth parameters, spore-induced inflammatory responses, that is, the production of cytokines (IL-1β, IL-6, TNFα) and NO, and cytotoxicity (CT) were compared using the PCA (Figure 1).

Stachybotrys chartarum

In PCA, the plasterboard 6b was positioned to the right with the positive score value along the PC1 axis, accounting for 46.3% of the variance (Figure 1B). On this board, after a weak growth with a slow growth rate and small biomass production, the spores of *S. chartarum* induced the greatest production of inflammatory mediators IL-1β, IL-6, TNFα, and NO, and caused moderate cytotoxicity in the RAW264.7 cell line (Table 1, Figure 1A).

The plasterboards 1a, 2a, 2b, 3a, 3b, 4a, and 4b formed one group close to zero score values along the PC1 axis (Figure 1B). The growth rates on these boards were slow, while the biomass production was intermediate, but varied so that boards 1a, 3a, 3b, and 4b supported growth better than boards 2a, 2b, and 4a. The spores induced low to moderate production of inflammatory mediators and were cytotoxic (Table 1, Figure 1A, B).

The plasterboards 1b, 5a, 5b, and 6a formed another group to the left along the PC1 axis with negative score values (Figure 1B). They supported good growth with a rapid growth rate and abundant biomass synthesis (Table 1, Figure 1A, B). There was one exception, the growth rate on board 1b was slow. The spores collected from boards 1b, 5a, 5b, and 6a induced low or moderate production of inflammatory mediators, whereas their cytotoxicity was among the highest. For *S. chartarum* a correlation was obtained between the growth rate and biomass synthesis ($r = 0.65$, $P < 0.05$).

Along the PC2 axis accounting for 27.1% of the variance, plasterboards 1a, 1b, 5a, 5b, 6a and 6b had positive score values (Figure 1B). Spores collected from these boards caused high or moderate cytotoxicity (Table 1, Figure 1A, B). In contrast, cytotoxicity caused

Table 1 Growth of *Stachybotrys chartarum, Aspergillus versicolor, Penicillium spinulosum* and *Streptomyces californicus* on front (a) and rear (b) sides of six different brands of plasterboards and spore-induced inflammatory responses and cytotoxicity in macrophages at the dose of 1×10^6 spores/10^6 cells

Microbe	Plasterboard	lag (d)	μ (%/d)	BM (%)	TNFα (ng/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	NO (μM)	CT (%)
S. chartarum	1a	5	4.1	80	2.7	2.6	1300	2.3	65
	1b	5	4.1	80	1.1	0.1	70	2.2	73
	2a	5	2.6	40	2.8	5.0	530	2.1	58
	2b	5	2.6	60	3.0	22	1200	2.2	65
	3a	5	5.1	80	2.6	1.1	250	2.5	47
	3b	5	5.1	80	2.6	2.0	800	2.6	54
	4a	8	4.1	60	2.8	10	210	1.8	42
	4b	8	5.0	80	3.2	3.2	1500	2.2	58
	5a	5	12	80	1.0	0.0	120	1.9	84
	5b	5	10	100	1.9	1.8	120	1.5	66
	6a	5	7.0	100	2.9	3.5	1900	2.4	79
	6b	5	3.4	60	4.3	290	3000	7.0	69
A. versicolor	1a	8	7.7	80	3.3	110	1700	2.6	65
	1b	8	3.5	60	3.5	500	2800	7.4	66
	2a	9	3.9	80	3.4	410	2800	3.9	66
	2b	9	2.6	60	3.5	520	2900	9.1	61
	3a	8	7.9	100	2.3	11	140	1.9	85
	3b	8	7.9	100	2.9	84	1100	1.6	77
	4a	8	5.4	80	2.2	18	82	1.3	74
	4b	8	5.1	100	2.4	21	140	1.4	65
	5a	8	7.5	60	2.9	47	670	1.5	65
	5b	8	7.5	60	2.0	97	910	1.5	76
	6a	20	3.0	60	3.4	580	2900	10	65
	6b	23	3.9	60	3.3	120	1900	2.0	80
P. spinulosum	1a	10	3.4	100	3.7	73	980	3.1	60
	1b	10	2.6	80	3.5	42	760	2.6	49
	2a	20	3.8	60	4.1	200	2400	4.4	69
	2b	13	1.6	40	4.0	180	2400	8.1	65
	3a	13	2.2	80	3.7	40	330	1.7	54
	3b	10	2.6	80	1.6	10	82	0.9	39
	4a	10	7.1	100	2.4	7.3	58	0.9	48
	4b	10	7.1	100	3.3	18	210	1.2	48
	5a	13	6.3	40	3.5	36	350	1.7	47
	5b	10	10	40	3.5	32	430	1.7	53
	6a	23	6.3	40	3.7	68	1100	2.2	50
	6b	15	10	40	3.5	78	660	2.9	56
S. californicus	1a	34	5.6	60	4.0	600	2700	14	72
	1b	34	5.6	60	3.5	930	2200	12	79
	2a	34	5.6	60	3.9	1400	2400	11	82
	2b	34	5.6	60	3.7	1400	2300	11	90
	3a	20	7.8	60	3.3	110	2100	3.2	40
	3b	20	7.8	60	3.2	350	2700	3.4	50
	4a	20	6.2	100	3.4	500	1800	4.1	61
	4b	20	6.2	100	3.1	130	2300	2.0	37
	5a	20	6.2	100	3.8	1100	2400	4.7	78
	5b	20	6.2	100	2.4	74	2400	1.2	33
	6a	20	6.2	100	4.0	840	2300	6.7	76
	6b	20	6.2	100	3.4	400	2400	6.1	83

Growth rates and biomass productions between different microbes with different morphologies cannot be compared as they were evaluated only visually.
lag = time needed for the beginning of growth, μ = growth rate, BM = biomass (percentage of the maximal amount of biomass achieved), TNFα = tumor necrosis factor alpha, IL-1β = interleukin-1 beta, IL-6 = interleukin-6, NO = nitric oxide, CT = cytotoxicity. Dose–responses of induced inflammatory responses and cytotoxicity are presented in detail in *Inhalation Toxicology*, **13**, 233–247 (2001).

by the spores collected from boards 2a, 2b, 3a, 3b, 4a and 4b with negative score values was among the lowest.

Aspergillus versicolor

The PCA separated plasterboards into two major groups according to the growth of *A. versicolor* and the inflammatory responses induced by the spores collected from the boards (Figure 1C, D). Along the PC1 axis, accounting for 62.9% of the variance, plasterboards 1b, 2a, 2b, 6a, and 6b were positioned to the right with positive score values. On these boards, *A. versicolor* grew weakly with a long lag phase (boards 6a and 6b), slow growth rate and only

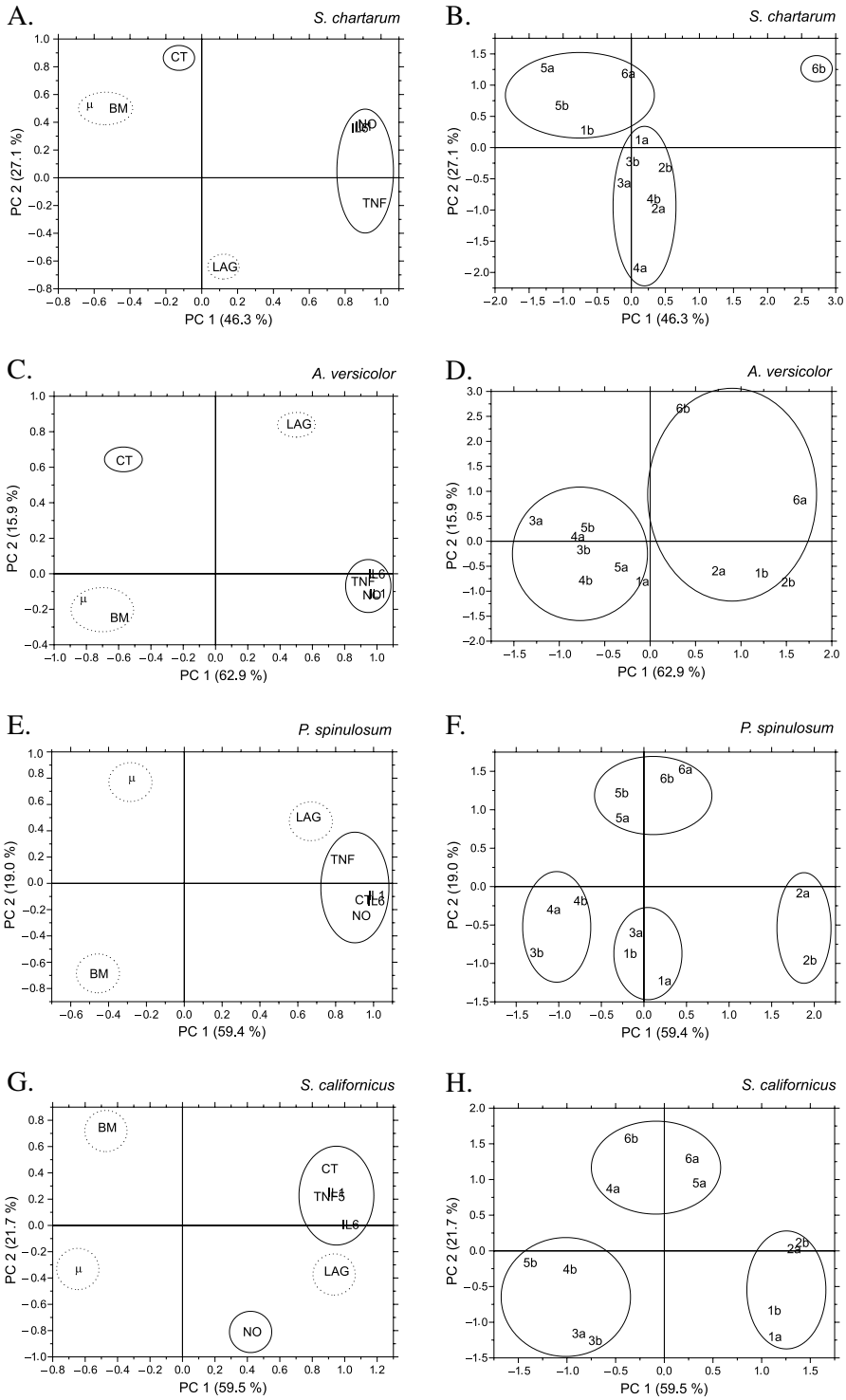

Fig. 1 Loading (A, C, E, and G) and score (B, D, F, and H) plots of PCA showing the separation of six different plasterboards from front (a) and rear (b) sides along the first two principal components (PC1 and PC2) using growth, inflammatory response, and cytotoxicity data of *Stachybotrys chartarum* (A, B), *Aspergillus versicolor* (C, D), *Penicillium spinulosum* (E, F), and *Streptomyces californicus* (G, H). Growth parameters are separated with dashed circles, and inflammatory responses, cytotoxicities, and plasterboards with solid circles. lag = time needed for the beginning of growth, μ = growth rate, BM = biomass, TNFα = tumor necrosis factor alpha, IL-1β = interleukin-1 beta, IL-6 = interleukin-6, NO = nitric oxide, CT = cytotoxicity

a small amount of biomass synthesized (Table 1, Figure 1C, D). The spores collected from these boards induced a major production of inflammatory mediators, whereas their cytotoxicity was quite low, with the exception of spores collected from board 6b, which were highly cytotoxic.

On the other group of plasterboards, positioned to the left along the PC1 axis, *A. versicolor* grew better (Table 1, Figure 1C, D). A high amount of biomass was produced with the slow (boards 4a and 4b) or fast (boards 1a, 3a, and 3b) growth rate. Alternatively, a low amount of mycelium was obtained but with a high growth rate (boards 5a and 5b). The cytotoxicity of spores collected from these plasterboards was among the lowest (boards 1a, 4b, and 5a) or high (boards 3a, 3b, 4a, and 5b). Concomitantly, they induced low or moderate production of inflammatory responses IL-1β, IL-6, TNFα, and NO in RAW264.7 cell line. The spore-induced NO ($r = -0.75$, $P < 0.01$), TNFα ($r = -0.58$, $P < 0.05$), IL-1β ($r = -0.79$, $P < 0.01$), and IL-6 ($r = -0.71$, $P < 0.01$) productions correlated negatively with the growth rate.

Along the PC2 axis, accounting for 15.9% of the variance, plasterboards 3a, 4a, 5b, 6a and 6b had positive score values (Figure 1D). On these boards the cytotoxicity of spores was among the highest recorded, except for spores from board 6a (Table 1, Figure 1C). In contrast, spores collected from plasterboards 1a, 1b, 2a, 2b, 3b, 4b, and 5a with negative score values were characterized by the lower cytotoxicity.

Penicillium spinulosum

The PCA separated plasterboards inoculated with *P. spinulosum* into four groups because of the differences in the growth and bioactivity of spores (Figure 1E, F). First, plasterboards 2a and 2b were found to the right with positive score values along the PC1 axis, explaining 59.4% of the variance in the data. On these boards *P. spinulosum* grew weakly with a long lag phase, a slow growth rate and poor biomass synthesis (Table 1, Figure 1E, F). The spores collected from these boards induced extensive production of NO, TNFα, IL-1β, and IL-6 and were among the most cytotoxic.

In contrast, plasterboards 3b, 4a and 4b were positioned to the left of PC1 axis with negative score values (Figure 1F), supporting the excellent growth with a short lag phase and abundant mycelium production (Table 1, Figure 1E). The spores collected induced only a low production of inflammatory mediators and had low to moderate cytotoxicity.

The plasterboards positioned close to zero score values along the PC1 axis formed two groups (Figure 1F). On boards 1a, 1b, and 3a the growth rate was slow and biomass synthesis great, in contrast to the high growth rate and low biomass production on boards 5a, 5b, 6a, and 6b (Table 1, Figures 1E, F). The spores collected from all of these boards caused moderate cytotoxicity and production of NO, TNFα, IL-1β, and IL-6 in mouse macrophages. The cytotoxicity of *P. spinulosum* correlated positively with the spore-induced NO ($r = 0.78$, $P < 0.01$), TNFα ($r = 0.77$, $P < 0.01$), IL-1β ($r = 0.90$, $P < 0.01$), and IL-6 ($r = 0.85$, $P < 0.01$) productions, in contrast to *S. chartarum* and *A. versicolor*.

Plasterboards 5a, 5b, 6a, and 6b had positive score values along the PC2 axis, accounting for 19.0% of the variance (Figure 1F), and characteristically supported the fast growth of *P. spinulosum*, although the ultimate amount of mycelium produced was small (Table 1, Figure 1E, F). The spores caused moderate cytotoxicity and inflammatory mediator production in RAW264.7 cell line. On the other boards with negative score values along the PC2 axis (Figure 1F), the growth rate was slow (1a, 1b, 2a, 2b, 3a, and 3b) or the biomass production high (boards 1a, 1b, 3a, 3b, 4a, and 4b) (Table 1, Figure 1E). The biological activity on these boards varied being low, moderate, or high.

Streptomyces californicus

The PCA separated plasterboards into three groups on the basis of *S. californicus* growth and the biological activity of the spores (Figure 1G, H). First, plasterboards 1a, 1b, 2a, and 2b were positioned to the right with positive score values along the PC1 axis, accounting for 59.5% of the variance in results. The growth of *S. californicus* on these plasterboards was poor, because of its very long lag phase and the small amount of biomass produced (Table 1, Figure 1G, H). The spores collected were among the most bioactive causing high or moderate cytotoxicity and the production of NO, TNFα, IL-1β, and IL-6 in RAW264.7 cell line. There was a negative correlation between lag phase and biomass synthesis ($r = -0.71$, $P < 0.05$).

In the second group, plasterboards 4a, 5a, 6a, and 6b were positioned close to zero score values along the PC1 axis (Figure 1H). They supported one of the best biomass productions of *S. californicus* after the shortest lag phase. Concomitantly, the spore-induced NO production was among the lowest detected. However, the spores induced high to moderate cytotoxicity as well as production of TNFα, IL-1β, and IL-6 (Table 1, Figure 1G).

In the third group, plasterboards 3a, 3b, 4b, and 5b were located to the left along the PC1 axis with negative scores (Figure 1H) supporting moderate (boards 3a, 3b) or excellent (boards 4b, 5b) growth (Table 1, Figure 1G), while the spore-induced NO production was moderate. The cytotoxicity and inflammatory responses IL-1β, IL-6, and TNFα induced by the spores collected from these plasterboards were among the weakest. Similarly as for *P. spinulosum*, positive correlations between cytotoxicity and induced TNFα ($r = 0.81$, $P < 0.01$), IL-1β ($r = 0.86$, $P < 0.01$), and IL-6 ($r = 0.85$, $P < 0.01$) productions were found for *S. californicus*.

Along the PC2 axis accounting for 21.7% of the variance in the results, plasterboards 4a, 5a, 6a, and 6b had positive score values (Figure 1H). On these

plasterboards, there was extensive biomass synthesis, whereas the spore-induced NO production was low (Table 1, Figure 1G, H). On the remaining boards the large amount of biomass production (boards 4b and 5b) was detected with spores inducing moderate NO production. In contrast, there were boards supporting a low amount of biomass, combined with spores inducing either high (boards 1a, 1b, and 3b) or moderate (boards, 2a, 2b, and 3a) NO production. A negative correlation ($r = -0.62$, $P < 0.05$) was obtained between biomass and spore-induced NO production.

Discussion

These results revealed that there were considerable differences in the growth of the studied microbes on the six different brands of plasterboards. In general, when the microbial growth on plasterboard was good, the spore-induced biological activity as measured by IL-1β, IL-6 and TNFα productions was low. In contrast, when the growth was weak, the spores contained compounds capable of inducing major cytokine responses in mouse macrophages. This inverse correlation explained the greatest variation in PCA results of all microbes studied along the PC1 axis, and has often been observed between the specific growth rate and the formation of secondary metabolites (von Döhren and Gräfe, 1997). Moreover, the spore-induced production of cytokines IL-1β, IL-6, and TNFα clustered together in PCA, indicating that they were dependent on similar aspects of the biological activity of the spores of these four microbes.

Stachybotrys chartarum grew faster than the other microbes on plasterboards. The *S. chartarum* spores collected from the plasterboards supporting the best growth also caused extensive cytotoxicity. This is an important finding with respect to the indoor air quality, since *S. chartarum* has a strong cellulolytic capacity and is usually found on the liners of heavily wetted plasterboards (Gravesen et al., 1994; Nikulin et al., 1994; Andersson et al., 1997). It produces several biologically potent mycotoxins such as trichothecenes (Nikulin et al., 1994; Nielsen et al., 1998a,b; Sorenson, 1999), which are carried along with *S. chartarum* spores (Sorenson et al., 1987; Pasanen et al., 1993; Nikulin et al., 1996, 1997; Rao et al., 2000). The mycotoxin production of *S. chartarum* is often associated with adverse health effects in occupants of mold problem buildings (Cooley et al., 1998; Hodgson et al., 1998; Johanning et al., 1999). The cytotoxicity of *A. versicolor* spores did not correlate with any particular growth conditions, but was observed both after poor and excellent growth. This characteristic may partly explain why *A. versicolor* is often associated with health problems in occupants of water-damaged buildings (Hodgson et al., 1998; Johanning et al., 1999). The cytotoxicities of *A. versicolor* and *S. chartarum* spores were not related with any inflammatory mediators induced in mouse macrophages. One possible reason for this observation is that the highly toxic mycotoxins produced by these molds may lead to acute necrosis, i.e., cell death, in the macrophages at an early time point of exposure. Altogether, these findings suggest that to avoid health problems related to *S. chartarum* and *A. versicolor*, prevention of their growth is necessary.

In contrast, cytotoxicity caused by the spores of *S. californicus* and *P. spinulosum* correlated with induced immunological responses of these spores. This indicates that the toxicity caused by these spores may be cytokine or NO mediated. Several studies have demonstrated that proinflammatory cytokines, e.g. TNFα and IL-1β or NO, can individually or in conjunction induce either apoptotic or necrotic cell death in many cell types, including mouse macrophages (Bonfoco et al., 1995; Beg and Baltimore, 1996; Brüne et al., 1997; Damoulis and Hauschka, 1997; Liu et al., 2000). The induced NO production by spores of *S. californicus* did not correlate with the induced cytokine productions. This is consistent with our earlier conclusion that some spore-derived capsule components, rather than metabolites of these gram-positive bacteria, may function as potent inducers of NO production (Hirvonen et al., 1997a).

The growth of the four microbes as measured by short lag phase, rapid growth rate or great biomass production was usually best on plasterboards 3 or 4, and poorest on board 2. In contrast, the biological activity of the spores collected from boards 3 and 4 was the lowest detected, whereas spores from board 2 were usually the most bioactive. Thus, plasterboards 3 and 4, which support good microbial growth with low bioactivity of the spores, possibly have similar nutrient compositions regulating microbial growth and secondary metabolism. The biological activity of the spores collected from boards 1, 5, and 6 was moderate or high, although the microbial growth on these boards was more strain-dependent. No major differences were observed in the growth between the front and rear sides of plasterboards. The only exception was the growth of *S. chartarum* on board 6, where the growth was markedly more intensive on the front side.

These results demonstrate that microbial spores serve as carriers of bioactive compounds produced by microorganisms. This is of special interest, because the spores of a large number of important fungi are sufficiently small in size to reach the alveoli where they can evoke respiratory symptoms. Furthermore, the bioactivity of spores can be regulated by different growth conditions provided by plasterboards. There was no particular plasterboard on which all the studied microbes would have grown poorly and yet shown a low biological activity. Nevertheless, there

was a promising trend in the clustering of plasterboards according to growth and bioactivity that can be used in the product development as a guide to help manufacturers produce microbiologically safer plasterboards.

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