



NAGase activity in airborne biomass dust and relationship between NAGase concentrations and fungal spores

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Abstract

Inhalation of airborne fungal spores or fungal enzymes may cause adverse pulmonary health effects. The enzyme NAGase (N-acetyl- β -D-glucosaminidase) is a chitinase presumed to be secreted by all fungi. In this study, NAGase activities and concentrations of fungi are estimated in airborne biomass dust to acquire knowledge about the level of NAGase activity and the relationship between NAGase activity and concentrations of airborne fungal spores. NAGase was sampled on both teflon and polycarbonate filters, and polycarbonate filters proved to be better for extraction of NAGase than teflon filters. NAGase was found in airborne dust at a biofuel plant and in dust generated from biomass. At a biofuel plant, the median level of exposure to NAGase was $21 \text{ pmol s}^{-1} \text{ m}^{-3}$. Significant correlations were found between NAGase activities, total number of fungal spores and CFU of fungi, with the highest degree of correlation being between the total number of fungal spores and the NAGase activity ($r = 0.802$, $n = 76$). Furthermore, when dust was stored for different periods, the culturability of fungal spores was strongly reduced and the NAGase activity was not or only slightly reduced after up to 40 days of storage. Accordingly, NAGase activity may be used as a rapid method to get an estimate of the exposure level to airborne fungal spores. Whether pure NAGase or the NAGase concentrations observed here cause any health effects is not known, although it has been shown that other fungal enzymes can cause respiratory disorders and a chitinase is described as an allergen.

1. Introduction

Inhalation of airborne fungal compartments, spores or enzymes may cause adverse pulmonary health effects. Valid methods for measuring levels of airborne fungi are crucial to research as well as to exposure assessment and risk evaluation. In some environments, large variations in exposure levels are observed within short time intervals (Levy et al., 1999). One way to overcome the problem of assessing exposure in environments with large variations in exposure levels is to sample by filtration and to use long sampling times. However, this requires that there is no effect of the flow present during filtration on the following quantification of the fungi. Airborne microorganisms are often quantified by counting, using microscopy

and/or cultivation (Fishwick et al., 2001; Näsman et al., 1999; Thorne et al., 1992; Würtz and Breum, 1997). However, both methods can underestimate the actual number of spores due to aggregation of spores, inadequate staining of spores and loss of viability due to flow on the sampling filter (Eduard and Aalen, 1988; Heldal, Skogstad and Eduard, 1996; Wang et al., 2001) and the fact that not all fungi are cultureable.

The general markers for fungal biomass, ergosterol and $\beta(1\rightarrow3)$ -glucans, and the extracellular polysaccharides of *Aspergillus* spp and *Penicillium* spp, have also been applied in studies of exposure to fungi (Douwes et al., 1999; Eduard et al., 2001; Szponar and Larsson, 2001). Instead of quantifying fungi or markers of fungi, exposure to particular toxic or allergenic constituents and metabolites may also be

determined. However, it is not known which fungal components cause respiratory problems. It has been shown that fungal $\beta(1\rightarrow3)$ -glucans may be aetiological agents in the relation between fungal exposure and adverse respiratory health effects (Douwes et al., 1996; Rylander, 1997; Thorn, Beijer and Rylander, 1998). However, in one study a weaker association between $\beta(1\rightarrow3)$ -glucans and work-related symptoms than between fungal spores and work-related symptoms was found (Eduard et al., 2001). Fungi produce extracellular enzymes, which can cause respiratory diseases (Houba et al., 1996; Jeffrey et al., 1999; Kauffman et al., 2000; Losada et al., 1986; Robinson et al., 1990) and a chitinase is described as an allergen (Blanco et al., 1999; Posch et al., 1999; Sanchez-Monge et al., 1999; Sowka et al., 1998). In spite of the respiratory effects that several fungal enzymes can cause, fungal enzymes are not quantified in studies of relations between exposure to fungi and adverse pulmonary health effects, for example. Thus, the concentrations at which fungal enzymes are present in airborne dust from different environments with high concentrations of airborne fungi, as in agricultural environments, are not known.

The fungal genera *Aspergillus*, *Alternaria*, *Penicillium*, *Trichoderma*, and *Verticillium* are often isolated from airborne dust in agricultural environments (Abdel-Hafez, Moubasher et al., 1990; Kotimaa et al., 1991; Nirenberg, Schmitz-elsheriz et al., 1995), and have been associated with respiratory disorders (Halpin et al., 1999; Kolmodin-Hedman et al., 1987; Schlueter, Fink et al., 1972). These fungi secrete the enzyme NAGase (N-acetyl- β -D-glucosaminidase) (Calonje et al., 1997; Clayessens and Ayerts, 1992; Draborg et al., 1995; Fenice et al., 1998; Hearn et al., 1998; Lahoz, Reyes and Leblic, 1976). Furthermore, NAGase is expected to be secreted by all chitin-containing fungi since chitin-degrading enzymes are thought to contribute to a number of morphogenic processes in filamentous fungi including spore germination, branch formation, and autolysis (Gooday et al., 1992; Lahoz, Reyes and Leblic, 1976). Thus, NAGase is expected to be present in airborne dust and quantification of NAGase may be a very rapid, alternative method for assessing exposure levels to fungal spores.

This paper reports studies of NAGase content in airborne dust derived from biomass. Relationships between the content of NAGase, CFU, and the counted microorganisms in airborne dust are examined. Furthermore, the effects of storage times of dust

on the NAGase content and the CFU of fungi are investigated.

2. Materials and methods

Dust from straw and wood chips (biomass) were derived by generation in a rotating drum and by collection of airborne dust from a biofuel plant.

2.1 Exposure measurement at a biofuel plant

The investigation was carried out on a Danish biofuel plant, where energy was gained from both straw and wood chips. In the morning and in the afternoon, seven and four cartloads straw were received, respectively. The measurements were performed on a working day in April, 2001. Inhalable bioaerosols were sampled (GSP samplers) for four hours in the morning and for four hours in the afternoon in different working areas. Furthermore, dust was sampled for eight hours side-by-side with the four hours samplers in nine of these areas. The dust-sampling instrument RESPICON (Hund Wetzlar, Germany) was used to measure airborne particles of the health-related sizes: respirable dust and inhalable dust. Personal dust monitoring was conducted using GSP samplers. The samplers were mounted with sterile teflon and polycarbonate filters for quantification of total numbers and CFU of fungi and NAGase activity and to identify which of the filters was the best for extraction of NAGase. Furthermore, teflon filters were used for endotoxin analysis. The dust was extracted the day after sampling. Results are presented as TWA (time weighted averages) measurements.

2.2 Generation of dust by the rotating drum

A rotating drum described Breum et al. (1997) was used for generating dust from biomass. The rotating drum consists of two parts including a dust generator and a dust sampler. A drum with a volume of 3.3 m³ was used as the dust generator and 3.0 kg of the straw (water percentage per weight 12–15%) or wood chips (water percentage per weight 32–53%) were loaded onto the bottom of the drum. The drum was rotated along the horizontal axis with a speed of seven rpm for a period of five minutes to generate dust and this dust was used for other purposes. The drum was then rotated again with the same test material and the same speed but for 10 min. At one end of the drum, a vacuum pump (420 l min⁻¹) was used to draw air

out of the drum, and the other end was connected to a filter to clean room air entering the drum. To analyse the dust generated for content of microorganisms and NAGase, up to six filter cassettes were used for sampling at a cross-section located 0.2 m from the outlet.

Dust was collected on sterile teflon filters placed in closed-faced field monitors (25 mm diam., 8 μm ; Millipore, Bedford, USA) and on sterile polycarbonate filters in closed-faced field monitors (25 mm diam., 0.4 μm , Nucleopore, Cambridge, MA, USA). Dust was generated from 34 straw samples and 22 wood chip samples of 3.0 kg and collected on a total of 148 filters. Of the 148 filters, 76 were used for studying the correlation between different microbiological factors, the other 72 were used in the storage experiment. Approximately one hour after its generation, the dust was extracted from the filters and part of the suspension immediately plate diluted.

In addition, a total of 14 filters with dust generated by the same rotating drum derived from experiments by Breum et al. (1999) were included in the correlation study. The dust in this experiment was derived from chopped straw, untreated or treated with lignosulfonate.

2.3 Extraction of dust

The dust on the teflon filters was extracted in 5.0 ml sterile 0.05% Tween 20 aqueous solution by orbital shaking (300 rpm) at room temperature for 60 min and centrifuged ($1000 \times g$) for 15 min, and the supernatant used for NAGase assay. In dust from the biofuel plant, the supernatant was also used for endotoxin assay. For the extraction of dust on polycarbonate filters, 5.0 ml sterile 0.05% Tween 80 and 0.85% NaCl aqueous solution was added to the filter cassette followed by a 15 min shaking period (500 rpm) at room temperature. The suspension was used for the following three analyses; total counts, CFU and, after centrifugation ($1000 \times g$, 15 min), for NAGase assays.

2.4 NAGase assay

NAGase activities were estimated according to Madsen and Neergaard (1999). To quantify the activity of NAGase (EC3.2.1.30), the release of *p*-nitrophenol from the substrate *p*-nitrophenol-N-acetyl- β -D-glucosaminide (Sigma Chemical Co. USA) was estimated. Culture filtrates (30 μl) were added to wells in micro-titre plates. Fifty μl of enzyme substrate solution (4 °C, 300 $\mu\text{g ml}^{-1}$ dissolved in

50 mm potassium phosphate, pH 6.7) was added to the wells. Appropriate controls without either the enzyme or the substrate were run simultaneously. The plates were incubated at 50 °C. Reactions were terminated and a yellow color developed following the addition of 50 μl 0.4 M Na_2CO_3 to each well. Absorbance was measured at 405 nm. One unit of enzyme activity is defined as the amount of enzyme, which releases 1 μmol of *p*-nitrophenol ml^{-1} enzyme min^{-1} . Activities are expressed as pmol s^{-1} per g dust or per m^3 air.

2.5 Quantification of microorganisms by a modified CAMNEA-method

Microorganisms were quantified by a modified CAMNEA-method (Palmgren et al., 1986). This method involves extraction of the aerosols on the filters followed by dilution and plating on different agar media. The number of fungi culturable on Dichloran Glycerol agar (DG 18 agar, Oxoid, Basingstoke, England) at 25 °C was counted. The number of bacteria culturable at 25 °C on Nutrient agar (Oxoid, Basingstoke, England) with actidione (cycloheximide; 50 mg l^{-1}), and the number of mesophilic actinomycetes (25 °C) on 10% Nutrient agar with actidione (50 mg l^{-1}) was estimated. The number of thermophilic actinomycetes culturable at 55 °C on 10% Nutrient agar with actidione (50 mg l^{-1}) was estimated.

The total numbers of fungal spores and bacteria were counted after staining in 20 ppm acridine orange (Merck). Fungi and bacteria were counted at a magnification of 1250 times by epi-fluorescence microscopy. The microorganisms were enumerated in forty randomly chosen fields or until a number of at least 400 were counted.

2.6 Endotoxin analysis by the LIMULUS method

The supernatant was analyzed (in duplicate) for endotoxin by the kinetic Limulus Amoebocyte Lysate test (Kinetic-QCL endotoxin kit, BioWhittaker, Walkersville, Maryland, USA). A standard curve obtained from an *Escherichia coli* O55:B5 reference endotoxin was used to determine the concentrations in terms of endotoxin units (EU) m^{-3} of air (12.0 EU \approx 1 ng).

2.7 Stability of NAGase and survival of fungi in dust on teflon filters

Dust generated from twelve biomass samples by the rotating drum collected on teflon filters was studied.

One filter with dust from each sample was used immediately for plate dilution for the quantification of mesophilic fungi and for NAGase activity. The other filters were incubated for 24 h at 22 °C and 55% rh (relative humidity) and the dust weighed. Subsequently, the filters were stored under the same conditions for up to 40 days. After storage, the filters were analysed for NAGase activity and CFU of mesophilic fungi.

2.8 Statistical analysis

Data were log transformed and the Pearson's correlation was calculated for the different measured parameters in SAS. Concentrations measured for each parameter in the morning and in the afternoon at the biofuel plant were log transformed and compared by Student's t-test. The effect of storage of dust on NAGase activity and CFU was analysed as t-test (LSD) in SAS, proc ANOVA.

3. Results

3.1 NAGase activities in airborne dust

To see whether NAGase can be detected in airborne dust and to get an estimate of the levels in an environment where straw and wood chips are handled, 59 air samples were taken and the concentrations observed are presented in Tables 1 and 2. NAGase activities were significantly higher in the afternoon than in the morning. In contrast, bacteria and endotoxin were present in higher concentrations in the morning, when more straw was received than in the afternoon (Table 2). In the straw receiving area, the respirable NAGase activity was estimated in two different locations and constituted 52% and 55% of the NAGase present in the inhalable dust fraction.

NAGase activity was found in all the dust samples generated by the rotating drum. In the 76 biomass dust samples, the NAGase concentrations were between 200 and 5×10^5 pmol s⁻¹ g⁻¹ dust (median = 1771 pmol s⁻¹ g⁻¹ dust; average = 21100 pmol s⁻¹ g⁻¹ dust). In the same dust samples fungi were found in concentrations between 5.3×10^5 and 5.4×10^{11} total spores/g dust (median = 1.0×10^9 ; average = 1.8×10^{10}) and 1.7×10^4 – 8.9×10^{10} cfu/g dust (median = 4.0×10^7 cfu/g dust; average = 3.1×10^9 cfu/g dust). In the dust generated from the 15 straw samples treated with the dust suppressant lignosulfonate, the

median NAGase activity was 2750 pmol s⁻¹ g⁻¹ dust (max = 4810 pmol s⁻¹ g⁻¹ dust).

3.2 Correlations between NAGase activity and other parameters in dust

The NAGase activity in the biomass dust and in the biofuel plant dust correlated significantly with CFU of fungi and with total number of fungal spores present in the same dust samples (Table 3). Similarly there was a significant correlation between CFU of fungi and NAGase activity in dust generated from lignosulfonate-treated straw (Table 3).

When dust was stored on teflon filters for periods of between 2 and 40 days, no significant correlation between the number of culturable fungi and the NAGase activity was found ($r = 0.212$) (Table 3). NAGase activity per total number of fungi and CFU of fungi per total number of fungi was calculated to see whether there was an association between the culturability of spores and the NAGase activity per spore, but no linear correlation ($r = 0.0057$, $P = 0.961$) was found.

3.4 Effect of storage time on NAGase and fungi content

When dust collected on teflon filters was stored for up to 40 days at 22 °C rh 55%, the NAGase activities (Figure 1) were slightly lower in 4 out of the 6 materials in the dust samples on the last day of estimation than on day zero. The numbers of culturable fungi were reduced significantly during storage of dust from all materials ($P < 0.001$) and mainly during the first days of storage (Figure 2). On day zero, on average 20, 4, 19, 9, 48 and 52 percent of the fungal spores were culturable from straw 1, 2, 3 and 4 and from chips 1 and 2, respectively.

Furthermore, dust was sampled for both 8 hours on one filter and for 4+4 hours on two filters. After 8 hours of constant sampling, the average NAGase activity per fungal spore was 1.22×10^{-5} pmol s⁻¹ m⁻³ and after 4+4 hours of sampling on two filters, it was 9.97×10^{-6} pmol s⁻¹ m⁻³. On average 18% and 30% of the fungal spores were culturable after 8 and 4+4 hours of sampling, respectively.

3.5 Extraction of NAGase from teflon versus polycarbonate filters

Dust was collected on both teflon and polycarbonate filters at 25 stationary positions at the biofuel plant and

Table 1. Concentrations of inhalable NAGase ($\text{pmol s}^{-1} \text{m}^{-3}$) in airborne dust at a biofuel plant at stationary positions (s) and as personal exposure (p). Concentrations are presented as intervals, and the median value is shown in parentheses.

Location of assessment	n	NAGase ($\text{pmol s}^{-1} \text{m}^{-3}$)	Total no. of fungal spores m^{-3}
Straw reception (s)	9	5.7–28.2 (18.3)	1.2×10^6 – 1.9×10^7 (8.8×10^6)
Straw store (s)	5	7.8–9.3 (8.4)	1.1×10^6 – 6.7×10^6 (2.3×10^6)
Chips store out side (s)	4	5.4–7.3 (6.3)	8.9×10^5 – 3.2×10^6 (9.9×10^5)
Office (p)	2	3.0–4.4 (3.6)	1.8×10^5 – 6.2×10^5 (4.0×10^5)
Working with straw reception (p)	4	13.3–18.6 (17.9)	2.1×10^6 – 9.7×10^6 (5.9×10^6)
Working with chips (p)	2	43.5–56.5 (50.0)	8.3×10^6 – 5.8×10^7 (3.3×10^7)
Observation at straw reception (p)	3	8.1–12.1 (11.7)	2.2×10^6 – 9.1×10^6 (4.1×10^6)
Outside reference (s)	2	2.2–3.4 (2.8)	1.8×10^4 – 2.0×10^4 (1.9×10^4)

Table 2. Concentrations of airborne inhalable NAGase and microorganisms (median of 15 values) in the morning and afternoon at a biofuel plant.

Exposure of	Morning	Afternoon
NAGase ($\text{pmol s}^{-1} \text{m}^{-3}$)	16.42 a*	27.87 b
Total no. of fungi m^{-3}	3.2×10^6 a	4.1×10^6 a
CFU of fungi m^{-3}	4.6×10^5 a	3.7×10^5 a
Actinomycetes m^{-3}	4.6×10^4 a	2.2×10^4 a
Total no. of bacteria m^{-3}	4.4×10^6 a	1.8×10^5 b
CFU of bacteria m^{-3}	1.8×10^5 a	6.4×10^4 b
Endotoxin EU m^{-3}	573 a	259 b

*Numbers in the same row followed by the same letter are not significantly different.

the NAGase concentrations in dust from both kinds of filters correlated well ($r = 0.956$, $P < 0.0001$, $n = 25$). The NAGase concentrations were, however, significantly higher ($P = 0.0023$) in dust extracted from polycarbonate filters (av. = $20.6 \text{ pmol s}^{-1} \text{m}^{-3}$ = $44336 \text{ pmol s}^{-1} \text{g}^{-1}$; median = $16.8 \text{ pmol s}^{-1} \text{m}^{-3}$ = $29738 \text{ pmol s}^{-1} \text{g}^{-1}$) than from teflon filters (av. = $10.3 \text{ pmol s}^{-1} \text{m}^{-3}$ = $27436 \text{ pmol s}^{-1} \text{g}^{-1}$; median = $7.9 \text{ pmol s}^{-1} \text{m}^{-3}$ = $20824 \text{ pmol s}^{-1} \text{g}^{-1}$).

4. Discussion

This study shows that NAGase could be detected in airborne dust at a biofuel plant and in dust generated from biomass. The exposure to NAGase was up to $56.5 \text{ pmol s}^{-1} \text{m}^{-3}$, and since no other papers report exposure to airborne NAGase, there is no data to compare these exposure levels with. Whether the NAGases present in airborne dust are involved

in the respiratory problems caused by fungi is not known, but studies in environments where materials containing fungal enzymes are handled indicate that α -amylase, protease, cellulase, phytase, and xylanase can cause respiratory disorders (e.g. Cullinan et al., 2001; Doekes et al., 1999; Houba et al., 1996; Jeffrey et al., 1999; Vanhanen et al., 2001). Furthermore, a chitinase is described as an allergen in plant food and latex (Blanco et al., 1999; Posch et al., 1999; Sanchez-Monge et al., 1999; Sowka et al., 1998).

The significant correlation between NAGase and total number of fungal spores indicates that fungal spores are the primary producers of NAGase in airborne dust and that quantification of airborne NAGase could be a rapid method to get an estimate of the level of fungal spore exposure. The correlations between NAGase and total number of fungi were 0.65 ($n = 44$) and 0.80 ($n = 76$). In agricultural dust, Eduard et al. (2001) found a correlation between $\beta(1 \rightarrow 3)$ -glucans and fungal spores on 0.67 ($n \cong 100$), and correlations between fungal spores and extracellular polysaccharides of *Penicillium* and *Aspergillus* were < 0.60 ($n \cong 60$). Douwes et al. (1999) found a correlation of 0.34 ($n = 55$) and of 0.45 ($n = 45$) between CFU of fungi and extracellular polysaccharides of *Penicillium* and *Aspergillus*. During storage of dust, the concentrations of culturable fungi were immediately reduced while NAGase activity was not, or only slightly, reduced. This is the cause for the observation of a lack of correlation between NAGase activity and CFU of fungi in the stored dust ($r = 0.212$). Furthermore, NAGase per spore and CFU of fungi per spore did not correlate well. These results confirm that the NAGase concentration present in airborne dust is not dependent on the culturability of the spores in the dust.

Table 3. Pearson's correlation (r) between NAGase activities and other microbial parameters. P-values are in parenthesis and n is the number of observations. Significant correlations appear in bold.

	NAGase	Total no. of fungi	CFU of fungi	Total no. of bacteria	CFU of bacteria	CFU of actinomycetes
NAGase	1.0	0.650	0.513	0.135	0.231	0.318
(Plant ¹ , n = 44)	(P = 0.0002)	(P = 0.0018)	(P = 0.559)	(P = 0.299)	(P = 0.139)	
Total no. of fungi	0.802	1.0				
(Biomass, n = 76)	(P < 0.0001)					
CFU of fungi	0.654	0.754	1.0			
(Biomass, n = 76)	(P < 0.0001)	(P < 0.0001)				
CFU of fungi	0.712	nt ²	1.0	nt	nt	nt
(Lignosul., n = 14)	(P = 0.0043)					
CFU of fungi	0.212	nt	1.0	nt	nt	nt
(Storage, n = 72)	(P = 0.260)					
Total no. of bacteria	-0.146	-0.081	-0.086	1.0		
(Biomass, n = 76)	(P = 0.220)	(P = 0.498)	(P = 0.472)			
CFU of bacteria	-0.068	-0.0004	0.260	0.104	1.0	
(Biomass, n = 76)	(P = 0.574)	(P = 0.997)	(P = 0.820)	(P = 0.385)		
CFU of actinomy	0.132	0.383	0.289	-0.086	0.0063	1.0
(Biomass, n = 61)	(P = 0.542)	(P = 0.451)	(P = 0.563)	(P = 0.474)	(P = 0.958)	

¹Designates the experiment the dust is derived from: Plant = airborne dust from biofuel plant; Biomass = dust generated from biomass by the rotating drum; Lignosul. = dust generated from lignosulfonate treated straw by the rotating drum; Storage = dust stored on filters up to 40 days.

²Not tested.

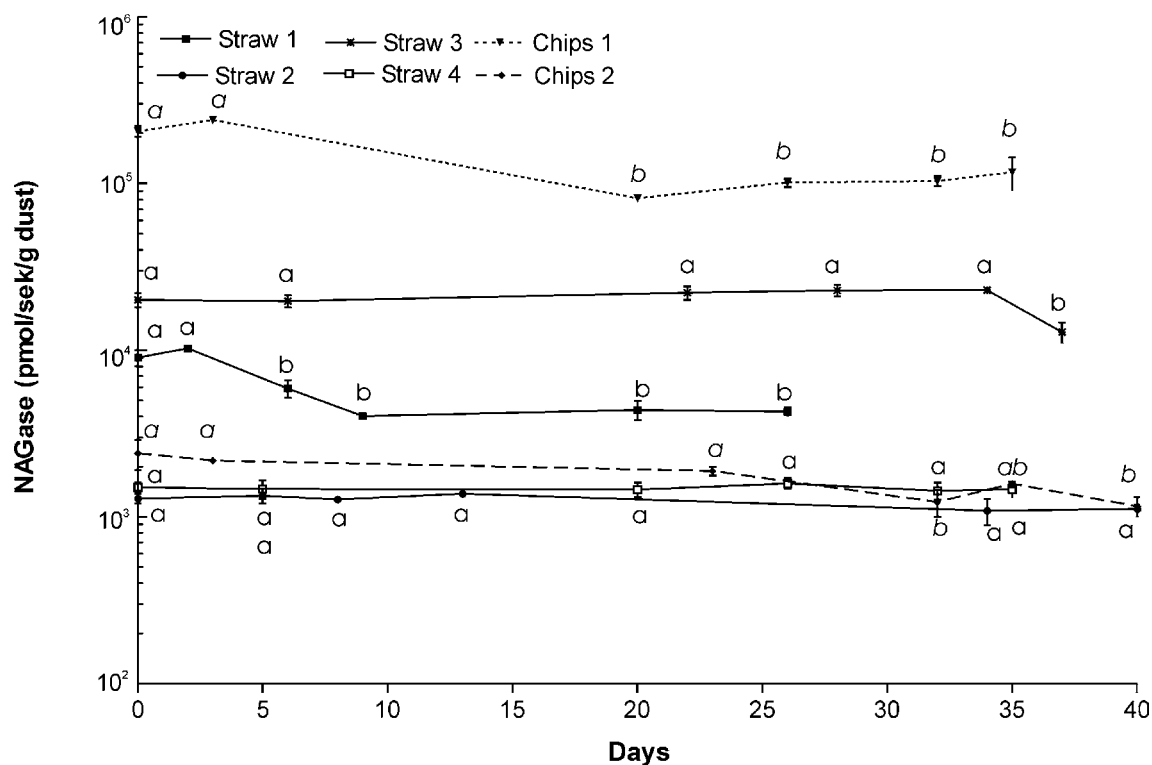


Figure 1. NAGase activity in dust as affected by days of storage at 22 °C, rh 55%. Points within each test material plot followed by the same letter are not significantly different.

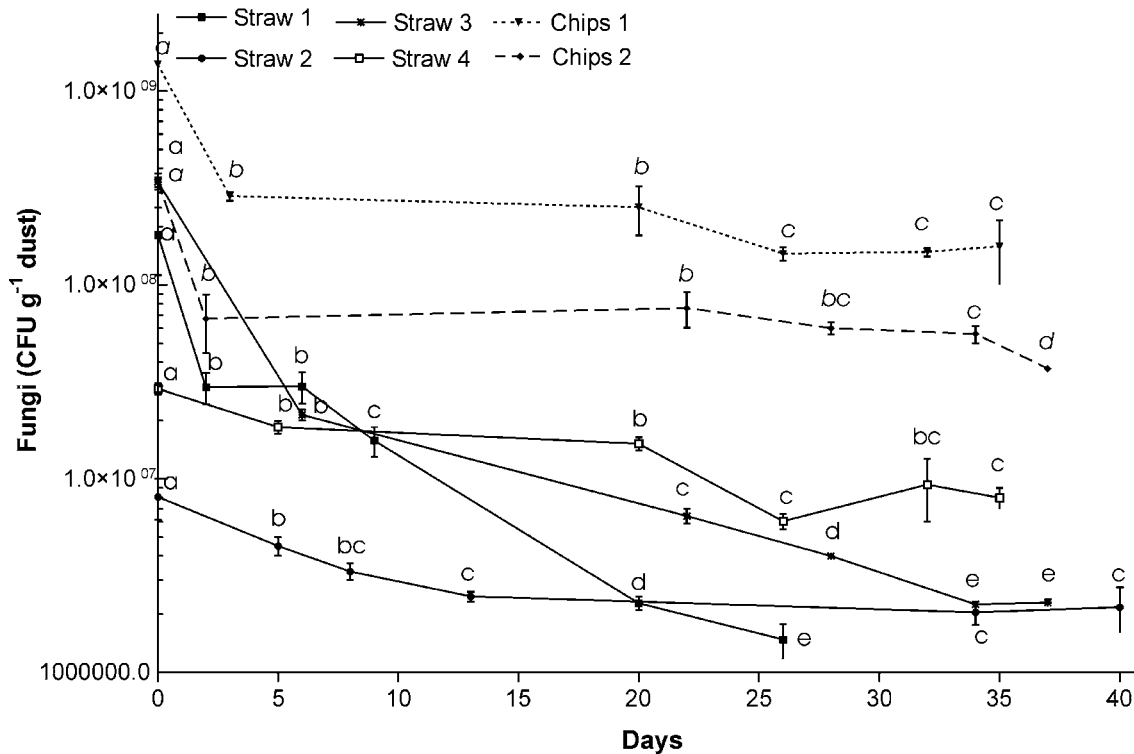


Figure 2. Number of culturable mesophilic fungi as affected by days of storage at 22 °C, rh 55%. Points within each test material plot followed by the same letter are not significantly different.

Accordingly, correlations were stronger between total numbers of fungal spores and NAGase activity than between CFU of fungi and NAGase activity in both experiments.

The airborne NAGase detected in this study is expected mainly to be associated with the airborne fungal spores. In the 76 biomass dust samples used for the correlation studies, the average NAGase activity per fungal spore was $3.9 \times 10^{-6} \text{ pmol s}^{-1}$, but a variation in NAGase activity per spore was seen, similar the content of ergosterol per fungal spore is known to vary (Miller and Young, 1997; Soares et al., 1995). The variation in NAGase activity per fungal spore may reflect an actual difference in NAGase activity per fungal spore, but it may also reflect the inaccuracy of the spore counting method described by Haldal, Skogstad and Eduard (1996) and Eduard and Aalen (1988).

NAGase activities were higher in the afternoon than in the morning. This may be because more NAGase was released in the afternoon than in the morning, but it may also be caused by an accumulation of NAGase in the air during the day, since the concentration of fungal spores was not significantly higher

in the afternoon than in the morning. If a portion of the NAGase accumulates in the air, this NAGase may have a lower sedimentation rate than fungal spores and consequently may be present as particles smaller than fungal spores or smaller than the clusters of spores the fungi may be present in. The observation that fungal spores did not accumulate in the air is in accordance with another study showing that the airborne conidia of *A. fumigatus* return to a background level within 1 h after a monitored activity cease (Passman, 1983). Furthermore, it is in accordance with the sizes of the studied inhalable fungal spores, thus the velocity of sedimentation of e.g. *A. fumigatus* conidia (diam. 2.5–3.0 μm (Domsch, Gams and Andersen, 1993)) is approximately 1 m h^{-1} (Gregory, 1973).

NAGase was sampled on both teflon and polycarbonate filters, and polycarbonate filters proved to be better for extraction of NAGase than teflon filters in this study. Consequently, polycarbonate filters may be preferred to teflon filters for NAGase sampling. However, as seen for endotoxin, greater suitability of filters for the extraction may also depend on the matrix in which the studied component is sampled (Douwes et al., 1995).

The reduction of fungal spore germination was high within the first days of storage, however, some spores were still culturable after 40 days of storage. This difference in resistance between spores is due to genus or species differences, but may also be due to the ages or physiological stages of the spores. These results are in agreement with observations showing different effects of storage on the culturability of *A. versicolor* and *P. commune* versus *P. variotii* spores sampled on filters (Näsman, Blomquist and Levin, 1999) and with results showing a loss of culturable fungi during storage of agricultural dust for 3 days (Thorne et al., 1994). In contrast, in a study about survival of fungi in settled indoor air dust, no effect of storage of dust for 25 days was seen (Macher, 2001). This difference between survival of fungi in biomass dust and in settled indoor air dust may partly be due to different characteristics of the two types of dust. It may also be due to the fact that the spores in the settled dust, in contrast to the spores from biomass, may have been airborne for one or more periods before the start of the study and consequently the spores most vulnerable to environmental exposure may already have lost their culturability. The observed reduction in survival of fungal spores collected on filters emphasizes the importance of shortening the period between sampling and plate dilution as much as possible. Furthermore, it is important to use a standard period between sampling and plate dilution to be able to compare studies where fungi are enumerated as CFU or are going to be identified. In contrast, when studying airborne NAGase, it is less important to have a short period between dust sampling and NAGase quantification since NAGase was stable under the conditions studied.

In conclusion, NAGase can be found in airborne dust and is consequently inhaled by humans, but whether the NAGase is involved in the health effects caused by inhalation of fungal spores is not known. NAGase activity correlated significantly with the total number of fungal spores and the NAGase activity was stable over time. The quantification of NAGase activity in airborne dust may be used as a rapid method to get an estimate of the exposure level to fungal spores.

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