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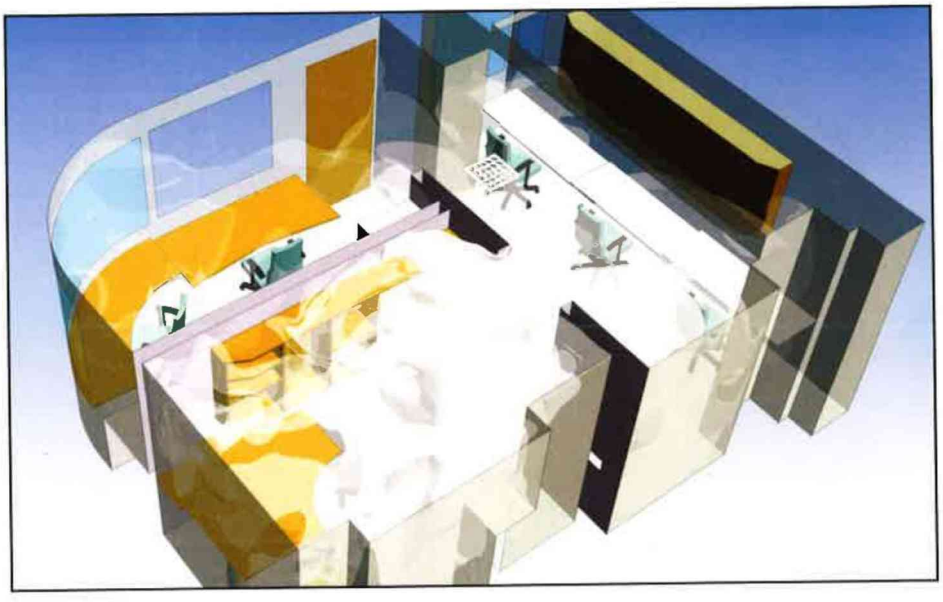

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INDOOR CHAETOMIUM-LIKE ISOLATES: RESISTANCE TO CHEMICALS, FLUORESCENCE AND MYCOTOXIN PRODUCTION

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ABSTRACT

Chaetomium-like fungi growing on indoor building materials produce toxic substances. Fourteen toxigenic indoor *Chaetomium*-like isolates from buildings in Finland were investigated. Six *Chaetomium globosum*-like strains from indoor dusts were toxic with boar sperm assay and cytotoxic to porcine kidney cells (PK-15), emitted green fluorescence and produced chaetoglobosins inhibiting cellular glucose transport. OT7 and OT7b strains from indoor dust were cytotoxic to PK-15 cells, non-fluorescent and produced the extremely cytotoxic protein synthesis inhibitor, chaetomin. The six *Chaetomium globosum*-like strains were resistant to borax and very sensitive to the wetting agent genapol used in cleaning chemicals. This may indicate that indoor *Chaetomium*-like fungi occupy their own ecological niche in buildings.

INTRODUCTION

The genus *Chaetomium* - especially the species *Chaetomium globosum* - is the most common representative of *Chaetomiaceae* in indoor environments. *Chaetomium* species are ubiquitous cellulolytic fungi producing over 500 bioactive substances when growing on various indoor building materials. The indoor growth of mycotoxin-producing *Ch. globosum* strains is an important condition connected to asthma in mold-infested buildings, furthermore *Ch. globosum* is also known as an important human pathogen. Other *Chaetomium*-like species found indoor have also been isolated from clinical samples. Knowledge about the diversity of indoor *Chaetomium*-like fungi, their ecology and metabolite production is therefore of great importance (Wang et al. 2016). Species diversity, toxicity towards mammalian cells, toxin production and biocide/chemical resistance of *Chaetomium*-like indoor isolates in Finland has not yet been investigated. The aim of this study was therefore to screen indoor *Chaetomium*-like isolates from buildings in Finland with 2 toxicity assays.

METHODS

Isolation of indoor strains, rapid toxicity screening and ethanol extraction of fungal biomass

Settled dust and inlet air filters were collected in urban and rural buildings where severe health problems were observed among humans and piglets.

Chaetomium-like isolates revealed toxicity in a rapid screening test done as described by Mikkola et al. /1/. These *Chaetomium*-like isolates were suspected to belong to *Chaetomiaceae* after the morphological examination of their ascomata /2/.

Isolation of the indoor microbial isolates and the ethanol-soluble compounds from biomasses of pure cultures grown on malt extract agar (MEA) were performed as described by Mikkola et al. /1/.

Identification of the fungal strains

Isolates were identified to the genus level based on the colony morphology on MEA, conidiophore morphology, the size of conidia and fluorescence abilities.

Chaetomium globosum MTAV 35, MTAV 37, *Aspergillus westerdijkiae* PP2, *Paecilomyces variotii* Pac2/kop and *Aspergillus versicolor* SL/3 strains were identified to species level in DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany).

DNA barcoding (www.isth.info) of internal transcribed spacer (ITS) sequences was used to identify the indoor *Trichoderma atroviride* strains.

Outdoor *T. atroviride* strains derived from the Szeged Microbiology Collection (www.szmc.hu, University of Szeged, Hungary).

Toxicity assays

The toxicity of ethanol-soluble compounds from biomasses was tested with boar sperm motility inhibition (BMSI), sperm membrane integrity disruption (SMID) and mammalian somatic cell toxicity (MSCT) assays as described by Bencsik et al. /3/, except that in BMSI the exposure was 20 min at 37°C.

Continuous cell line PK-15 (porcine kidney cells) and a malignant cell line MNA (murine neuroblastoma cell line) provided by EVIRA (Helsinki, Finland) were used in the MSCT assay.

Analysis of the mycotoxins

Analysis of the mycotoxins contained in the ethanol-soluble compounds of biomasses of the indoor *Chaetomium*-like strains was performed with LC-MS as described by Mikkola et al. /1/. Pure mycotoxins were purchased from Sigma-Aldrich Finland.

Toxicity of biocides and the wetting agent genapol towards fungi

The toxicity of biocides and chemicals towards indoor *Chaetomium*-like isolates and the indoor reference strains of *Aspergillus, Paecilomyces* and *Trichoderma atroviride* was assessed with the inhibition of fungal spore germination (shown on fig. 1B), with spore suspensions of 10^6 fungal spores ml^{-1} as described by Chitarra /4/. The test was performed on microtiter plates as described by Bencsik et al. /3/ for the MSCT assay. The formation of germ tubes was inspected by phase contrast microscopy after 1 and 2 d of incubation at 28°C. The EC_{50} corresponded to the inhibition of 50% of the conidia compared to the ethanol control (1 %). The tests were run in triplicate and calibrated with triclosan giving a mean SD ±< 20 %. The biocides and the wetting agent genapol were purchased from Sigma-Aldrich Finland, while the fungicide Boracol x10RH was from a local supplier.

RESULTS

Characterisation of the *Chaetomium*-like isolates

Table 1 shows the size of ascospores and the fluorescence profiles of the ethanol-soluble compounds from the biomass of the Chaetomium-like isolates. The morphology of their

ascomatal hair (Fig. 1A) was also used for characterization. The isolates were categorized into green, blue, yellow or no fluorescence emitted.

Table 1. Characterization of the Chaetomium-like isolates.

Isolate	Isolated from	Substratum	Fluorescence	Size of ascospores (µm)
MTAV 35	Public building, Oulu	Settled dust	green	10.6 × 9*
MTAV 37	Public building, Oulu	Settled dust	green	10.5 × 9*
MH1	Public building, Espoo	Settled dust	green	8.5 × 7.6*
RUK 10	Apartment, Helsinki	Settled dust	green	10.4 × 8.8*
ABCD	Apartment, Helsinki	Settled dust	green	9 × 7.6*
MO9	Piggery, Orimattila	Bedding	green	10.3 × 7.6*
2c/MT	Apartment, Vantaa	Settled dust	green	9.5 × 7.3*
MO15	Piggery, Orimattila	Bedding	yellow	12 × 8**
Ch1/tu	Public building, Espoo	Inlet air filter	blue	5.7 × 4.1**
OT7	Office, Helsinki	Settled dust	none	8.9 × 7.8*
OT7b	Office, Helsinki	Settled dust	none	9 × 7.7*

*Ascomatal hair coiled, unbranched. ** Ascomatal hair dichotomously branched

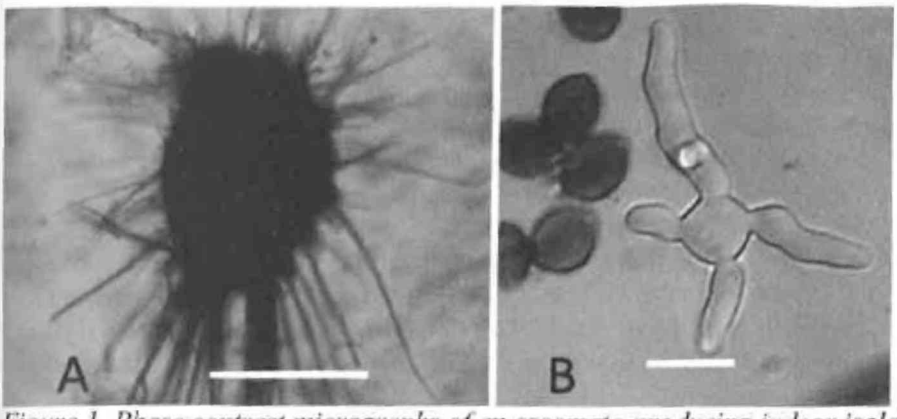

Figure 1. Phase contrast micrographs of an ascomata-producing indoor isolate. A) Formation of ascomata typical of Chaetomiaceae (bar 100 µm). B) Ascospores and formation of a germ tube indicating spore germination (bar 10µm).

Toxicity of biocides and genapol towards indoor *Chaetomium*-like isolates

The fluorescent isolates shown in Table 2 were tested for their resistance to biocides, chemicals and genapol (a wetting agent used indoors). The fungal isolates were more resistant to genapol and the biocides than the mammalian cell lines PK-15 and MNA. The green-fluorescent isolates were 4-fold more resistant to borax than the *Trichoderma atroviride* reference strains. The green-fluorescent isolates were quite sensitive to the other biocides compared to the other reference strains. Only the green- and blue-fluorescent isolates were over 1 000 times more sensitive to genapol than the yellow-fluorescent isolate and the reference strains. The blue-fluorescent isolate was also sensitive to borax. On the contrary the yellow-fluorescent isolate was resistant to both genapol and borax, similarly to the *Aspergillus* and *Paecilomyces* strains.

Table 2. Toxicities of biocides and the wetting agent genapol to Chaetomium-like strains compared with selected in- and outdoor fungal strains and mammalian cell lines.

	EC_{50} ($\mu g.ml^{-1}$)						
	Borax	Boracol	PHMB	Genapol	Fenoxy-ethanol	Chloramine	Triclosan
Indoor *Chaetomium*-like strains							
Green-fluorescent strains[a]							
	≥5000	100	4-8	<50	700-1500	1200-2500	2-4
Blue-fluorescent strain[b]							
	1200	100	4	<50	1500	1200	2
Yellow-fluorescent strain[c]							
	5000	100	4	50000	1500	1200	2
Mammalian cell lines (MSCT assay)							
MNA	150	<50	4	25	400	80	4
PK-15	600	<50	15	25	1500	150	15
Reference strains							
Indoor *Trichoderma atroviride* isolates [d]							
	1200	100	4	>50000	1500-3000	1200	16-30
Outdoor *Trichoderma atroviride* isolates [e]							
	1200	100	4-16	>50000	1500-3000	1200-2500	8-16
Indoor *Aspergillus* isolates [f]							
	≥5000	400-800	30-60	>50000	1500-3000	600-1200	8-16
Indoor *Aspergillus* isolates [g]							
	5000	100	30-60	>50000	800-400	600-1200	16
Indoor *Paecilomyces* isolates [h]							
	≥5000	400	30	>5000	3000	1200	4

[a] MTAV 35, MTAV 37, MH1, RUK 10, ABCD, MO9, 2c/MT; [b] Ch1/tu; [c] Mo15; [d] Ke14, Kiv10, Tri335, 14/AM; [e] SZMC 12323, 12474, 12495, 12541, 1723, 207080; [f] growing at 37°C: 32/skk, 33b/skk, 7D /skk, 1/skk, Asp21/skk; [g] not growing at 37°C: *A. westerdijkiae* PP2, AW/KL, *A.versicolor* SL/3; [h] growing at 37 °C: *P. variotii* Pac2/kop, Pac/skk, Pac /his.

Metabolite and toxicity profiling of *Chaetomium*-like isolates

The toxicity to mammalian cells and metabolite profiling of the green- and non-fluorescent strains is shown in Table 3. The green-fluorescent strains MTAV 35, MH1, RUK 10 and ABCD containing chaetoglobosins were more toxic in the sperm assays than in the MSCT. Pure chaetoglobosin A and the chaetoglobosin-containing extracts had > 10-fold higher EC_{50} concentrations in the SMID assay than in the BMSI assay. This indicates that chaetoglobosins and chaetoglobosin-containing extracts immobilized sperm cells but did not disrupt the sperm plasma membrane like the lethal toxin alamethicin. The 2c/MT strain excreted chaetoglobosins in the exudates. The ethanol-soluble compounds from the biomass of non-fluorescent strains were 100-fold more toxic in MSCT than those of the chaetoglobosin-producing isolates. The ethanol-soluble compounds from the biomass of non-fluorescent strains were more toxic in the MSCT than in the BSMI and SMID assays, similarly to the protein synthesis inhibitor sterigmatocystin. The protein synthesis inhibitor chaetomin was detected in the ethanol-soluble compounds from the biomass of the non-fluorescent strains.

Identification of *Chaetomium*-like isolates to the species level

Strains MTAV 35 and MTAV 37 were identified as *Ch. globosum*. The other green-fluorescent isolates (MH1, RUK 10, ABCD and 2c/MT) are probably belonging to the same species as MTAV 35 and MTAV 37, as they also produce chaetoglobosins and chaetoviridin, and have similar ascomatal hair and size of ascospores. The difference between OT7 and OT7b strains and *Ch. globosum*-like isolates were the chaetomin production, extreme cytotoxicity and the absence of fluorescence.

Table 3. Toxicity- and metabolic profiling of the ethanol-soluble compounds of biomasses of the non-fluorescent and some green-fluorescent isolates.

	EC_{50} of the ethanol-soluble compounds from biomasses ($\mu g.ml^{-1}$)				
Strain	BMSI 20 min	SMID 2h	MSCT 2d	Identified metabolite	Estimated concentration mg/ml [a]
Ch. globosum MTAV35	5	450	40	Chaetoglobosins	3.4
				Chaetoviridin A	0.02
				Chaetoviridin C	0.2
Ch. globosum MTAV37	10	350	30	No data	
MH1	5	310	50	Chaetoglobosins	3.9
				Chaetoviridin A	0.5
				Chaetoviridin C	0.2
RUK 10	5	300	20	Chaetoglobosins	4.2
				Chaetoviridin A	0.04
				Chaetoviridin C	0.05
ABCD	5	450	30	Chaetoglobosins	4.24
				Chaetoviridin A C	0.3
				Chaetoviridin C	0.05
2c/MT				Chaetoglobosins[b]	
OT7	10	480	0.5	Chaetomin	1.3
				Chaetoviridins	0.13
				Chaetoviridin C	0.02
OT7b	10	480	0.8	Chaetomin	1.2
				Chaetoviridin A	0.3
				Chaetoviridin C	0.2
Commercial pure mycotoxins *				Biological activity	
Alamethicin	5	1	8	K^+ and Na^+ ion channel former	
Chaetoglobosin A	1	12	2	Inhibitor of glucose transport	
Citrinin	>100	50	10	Cytotoxic, nephrotoxic	
Sterigmatocystin	>20	>20	0.5	Inhibitor of protein synthesis	

[a] Amount of compounds in the methanol soluble compounds calculated from the total absorbance (220 nm) of the HPLC chromatograph.
[b] Chaetoglobosins found in exudates from MEA plates * Sigma-Aldrich

DISCUSSION

Although the number of indoor isolates in this study was limited, the results indicate that chaetoglobosin-producing *Ch. globosum*-like strains represented the most common but not the only ascomata-producing fungi in the investigated Finnish buildings. Wang et al. /2/ reported that the most commonly isolated indoor *Chaetomium* species worldwide is *Ch. globosum*.

The blue-fluorescent CH1/tu isolate possibly originating from outdoor air was more sensitive to borax than the other indoor *Chaetomium*-like isolates, indicating that indoor *Chaetomium*-like isolates may occupy their own ecological niche in buildings.

Chaetomin-producing indoor isolates were not reported in Finland earlier.

The blue-(CH1/tu) and yellow-fluorescent (MO15) isolates had dichotomously branched ascomatal hair like the members of the genus *Dichotomopilus* which is separated from the genus *Chaetomium* (Wang et al 2016).

Fluorescence, biocide/genapol resistance, as well as toxicity- and metabolite profiling may be useful in preliminary tracking of diversity in the case of indoor ascomata-producing *Chaetomium*-like isolates.

The results demonstrate species variability in biocide/chemical resistance among indoor fungal species and genera. The indoor use of biocides and chemicals may influence proliferation and species diversity of the indoor microbiota.

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