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Differential Chlorate Inhibition of *Chaetomium globosum* Germination, Hyphal Growth, and Perithecia Synthesis

Charles L. Biles · Desiree Wright · Marianni Fuego · Angela Guinn · Terry Cluck · Jennifer Young · Markie Martin · Josiah Biles · Shubhra Poudyal

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Abstract *Chaetomium globosum* Kunze:Fr is a dermatophytic, dematiaceous fungus that is ubiquitous in soils, grows readily on cellulolytic materials, and is commonly found on water-damaged building materials. Chlorate affects nitrogen metabolism in fungi and is used to study compatibility among anamorphic fungi by inducing *nit* mutants. The effect of chlorate toxicity on *C. globosum* was investigated by amending a modified malt extract agar (MEA), oat agar, and carboxymethyl cellulose agar (CMC) with various levels of potassium chlorate ($KClO_3$). *C. globosum* perithecia production was almost completely inhibited (90–100 %) at low levels of $KClO_3$ (0.1 mM) in amended MEA. Inhibition of perithecia production was also observed on oat agar and CMC at 1 and 10 mM, respectively. However, hyphal growth in MEA was only inhibited 20 % by 0.1–100 mM $KClO_3$ concentrations. Hyphal growth was never completely inhibited at the highest levels tested (200 mM). Higher levels of $KClO_3$ were needed on gypsum board to inhibit perithecia synthesis. In additional experiments, $KClO_3$ did not inhibit *C. globosum*, *Fusarium oxysporum*, *Aspergillus niger*, *Penicillum expansum,* and airborne fungal spore germination. The various fungal spores were not inhibited by $KClO_3$ at 1–100 mM levels. These results suggest that *C. globosum* perithecia synthesis is more sensitive to chlorate toxicity than are hyphal growth and spore germination. This research provides basic information that furthers our understanding about perithecia formation and may help in developing control methods for fungal growth on building materials.

Keywords *Chaetomium* sp. · Fungal inhibition · Mold · Potassium chlorate · Chlorate toxicity · Spore germination

C. L. Biles (✉) · D. Wright · M. Fuego · A. Guinn · T. Cluck · J. Young · M. Martin · J. Biles · S. Poudyal
Department of Biology, East Central University, 1100 East 14th Street, Box N-5, Ada, OK 74820, USA
e-mail: cbiles@ecok.edu

Introduction

Indoor air quality is a major health concern in public buildings and homes. Molds (fungi) have been implicated as primary allergens in homes that can cause a variety of human respiratory problems [5, 13, 17, 23, 27]. Indoor mold of greatest concern is directly associated with water-damaged building materials [4, 17]. Approximately 30 % of the homes in America and Europe have excess moisture problems that allow considerable mold growth [24]. The most common symptom of a mold disorder is the allergic response due to IgE antibody production because of exposure to fungal hyphae, spores, and proteins [7, 17].

Most members of the Chaetomiaceae are cellulolytic and grow on a variety of carbon sources including building materials, paper, and cotton fabrics [1].

C. globosum Kunze:Fr is a fungus placed in the Phylum Ascomycota [1] and is in the Family Chaetomium. *C. globosum* is identified by the dark ostiolate perithecium with numerous hairs extending from the perithecium [20] that can be visualized on wall board (Fig. 1). The ascospores within the perithecia are the primary agents of dissemination (Fig. 1). Both hyphae and fungal spores can be allergenic. In rare cases, *C. globosum* has been associated with phaeohyphomycosis and opportunistic infections [2, 19], dermatomycoses [38] contamination of dialysis fluid [14], onychomycosis [21], and infection of dystrophic nails [36]. Mycotoxins of *C. globosum* have been characterized [15, 16] and found toxic to laboratory animals [29]. In addition, Wijeratne [40] has shown that *C. globosum* synthesizes anti-cancer compounds.

Chaetomum globosum is commonly found as a saprophyte on straw and dung [1]. Because of the ability to grow on cellulose- and lignin-rich substrates, *C. globosum* has been investigated as a potential biocontrol against several other plant pathogenic fungi [10, 26]. Strains of *C. globosum* have shown competition for the cellulolytic substrates in which the plant pathogens often reside and have exhibited antibiosis [11] and mycoparasitism. As a cellulolytic fungus, *C. globosum* is known for discoloring paper in books and other artifacts [32, 37]. It is commonly found on building materials that have been exposed to water [3, 22, 33].

Sensitivity of fungi to chemicals in the form of fungicides and disinfectants is well known. Chlorate is a nitrate analogue and interferes with nitrate reduction to ammonium and has been used to study nitrate assimilation in fungi, bacteria, and algae [12, 39]. Others postulate that chlorate is converted to chlorite by nitrate reductases and the toxic compound is chlorite [39]. Cove [9] found that chlorate toxicity affecting *Aspergillus nidulans* was caused by the interference of chlorate with the breakdown of organic sources that would provide nitrogen to the organism. Preliminary studies in our laboratory indicated that chlorate was toxic to *C. globosum* perithecia production at very low concentrations on both media and gypsum board [42]. By stimulating nitrate nonutilizing (*nit*) mutants with $KClO_3$, vegetative compatibility and relatedness may be determined. It has been used to test for vegetative compatibility among *Fusarium oxysporum* isolates [8, 31]. According to van Wijk [39], chlorate is nontoxic to most aquatic organisms, except for a small group of marine brown algae.

Our laboratory is interested in the basic biology of fungi and factors that affect fungal growth and reproduction. In the process of screening several chemicals to determine their effect on *C. globosum*, we discovered that $KClO_3$, utilized in stimulating *nit* mutants, inhibited perithecia production. The purpose of this study was to determine the amount of spore germination, hyphal growth, and perithecia synthesis by *C. globosum* when exposed to various concentrations of potassium chlorate ($KClO_3$). This information will increase our basic knowledge of perithecial growth of *C. globosum* and may provide useful information to help control the growth of this fungus in indoor environments. Hyphal growth and perithecia synthesis were observed on several media including MEA, CMC, and oat agar using various isolates of *C. globosum.* Spore germination was observed on MEA amended with $KClO_3$, and *C. globosum* spore

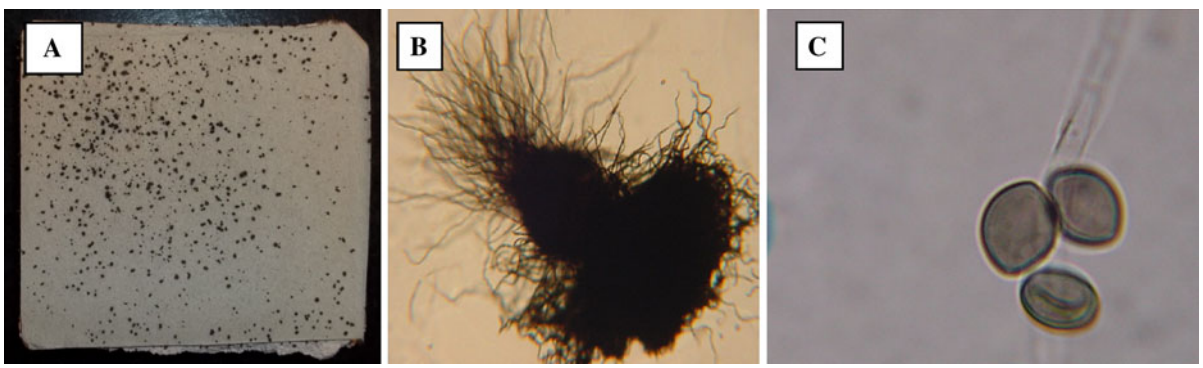

Fig. 1 *Chaetomium globosum* perithecia on; **a** perithecia on gypsum board, ×1, 250 μm diameter; **b** perithecium (×100, 250 μm diameter); **c** ascospores (×400, 8–11 × 6–8 μm)

germination was compared with other fungi commonly found in water-damaged buildings. Perithecia synthesis of *C. globosum* on MEA was compared with perithecia synthesis on gypsum board, where *C. globosum* often grows in water-damaged buildings.

Materials and Methods

Obtaining and Maintaining the Fungus

Chaetomium globosum isolates were obtained from various sources. *C. globosum* ECU 1490 and ECU SC-1 were isolated from healthy Cat Brier (*Smilax* sp.) leaves in 2008. The NM isolate was obtained from a contaminated piece of gypsum board from a water-damaged house in New Mexico compliments of Dr. Geoffrey Smith, New Mexico State University, Department of Biology, Las Cruces, New Mexico, USA, in 2005. The NMmt isolate was obtained from an inoculated piece of gypsum board at ECU that appeared to have different growth patterns than the NM isolate. PI-932 was obtained from Presque Isle Cultures, Inc., and the CB isolate was obtained from Carolina Biological Company. The ECU-lib *C. globosum* isolate was obtained from water-damaged carpet at the East Central University Library in Ada, Oklahoma. *Aspergillus niger*, *Penicillium expansum*, and *F. oxysporum* isolates used in the germination studies were obtained from Presque Isle Cultures, Inc. All isolates were grown in pure culture and maintained on malt extract agar or potato dextrose agar (PDA).

The fungal isolates were grown on media plates by taking a plug of the fungus from the malt extract agar (MEA). The 5-mm-diameter plug was placed in the center of the plate with the hyphae side of the plug against the media surface.

Comparison of Growth on Various Media

The fungus, *C. globosum*, was grown on several types of media to determine which was best for visualization and quantification of perithecia. Potato dextrose agar (PDA) and cornmeal agar (CMA) were from Fisher Scientific (Pittsburg, PA) and BBL™ (Becton, Dickinson and Co., Sparks, MD), respectively. These media were prepared according to the label directions. Materials for the other media are as follows: carboxymethylcellulose (CMC): 10 g carboxymethylcellulose sodium salt (Sigma cat no. C5678), 1 g NH_4NO_3, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 1 g yeast extract, and 12 g agar/L DI water; Czapek-Dox agar (CZ): 3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, $FeSO_4 \cdot 7H_2O$, 30 g sucrose, 15 g agar/L (Difco™, Becton, Dickinson and Co., Sparks, MD); oat agar (oat) was prepared from whole toasted oats (60 g) from a local grocery store (Walmart, Bentonville, AR), and 600 mL DI water was placed in a Waring blender and homogenized for 5 min. After homogenization, 400 mL DI water and 15 g Difco agar/L were added prior to autoclaving; rice agar (RM): 20 g of rice was homogenized in 600 mL of DI water, 400 mL of DI water; 15 g of Difco agar was added to the slurry before autoclaving; malt extract agar (MEA): 8 g Bacto ™ malt extract (Bacto ™, Becton, Dickinson and Co. Sparks, MD) and 15 g agar/L DI water; SNA: 1 g K_2HPO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 KCl, 0.2 g sucrose, 0.2 g glucose, and 20 g Difco agar/L of DI water; quarter strength PDA (QPDA): 6 g PD broth media (Difco ™, Becton, Dickinson and Co., Sparks, MD) and 15 g Difco agar/L of DI water. All media were autoclaved for 15 min at 121 psi and cooled, before pouring into 90-mm Petri dishes. All inoculated media were incubated in the dark at room temperature (25 °C). The optimum temperature for *C. globosum* growth was 25–30 °C. Growth was completely inhibited at 5 and was reduced in most isolates at 33 and 37 °C (Tables 2, 3). Preliminary experiments indicated that light may inhibit sporulation [43]. Therefore, all treatments were placed in a 24-h dark growth chamber or covered in aluminum foil at room temperature.

Comparison of *Chaetomium globosum* Growth Incubated at Different Temperatures

Initial experiments were conducted to determine the optimum temperature for growth and perithecia synthesis. Six *C. globosum* isolates were inoculated onto PDA plates as described above. The plates were then placed in 5, 25, 30, 33, or 37 °C incubators. Diameter was measured every 7 days, and perithecia incidence recorded after 14 days. The experiment was repeated with MEA and five isolates at 25 and 30 °C. Growth was measured every 7 days, and incidence of perithecia recorded after 14 days.

Growth in Malt Extract Broth (MEB) with Varying Amounts of Potassium Chlorate

Studies were conducted to determine whether potassium chlorate inhibited *C. globosum* (NM isolate) growth in malt extract broth (MEB: 8 g of malt extract per 1,000 mL DI water), when the MEB was amended with differing amounts (0–122 mM) of potassium chlorate. Upon removal from the autoclave, the pH of each broth was standardized at 5.5 using either 1 M NaOH or 1 M HCl. Three flasks of each potassium chlorate level containing 50 mL of broth were inoculated with 2 plugs cut from a plate of *C. globosum* grown on MEA.

The plugs were grown in the broth for 2 weeks on a platform shaker rotating at 50 rpm at 25 °C (RT). The hyphal strands were separated from the broth using a Büchner funnel with Whatman #1 filter paper that was placed in a side arm flask and a vacuum attached to the side arm. The filter paper was moistened and weighed before filtration, and then weighed immediately after filtration.

Growth on Solid Media Amended with Varying Levels of Potassium Chlorate

Chaetomium globosum (NM isolate) was grown on MEA and oat agar containing various amounts (0–15 g/L; 0–122 mM) of $KClO_3$-amended MEA. A fungal disc (5 mm) of *C. globosum* was removed from an actively growing edge of a 7- to 14-day MEA culture plate and placed in the middle of a $KClO_3$ amended or control plate (0 mM $KClO_3$). Four to five replications for each treatment were grown for 21 days at 25 °C. The plates were measured every 7 days for hyphal growth, and the number of perithecia was counted after 21 days of growth. Perithecia were counted using a bacterial colony counter magnified approximately 10×. When the density of the perithecia was too high on a single plate (>300), the perithecia on a quadrant of the plate was counted and this figure was used to estimate the total number of perithecia per plate. Due to the high number of perithecia formed on oat (Fig. 2), 10 mL of sterile DI water was placed on each oat Petri dish after 14–21 days of growth. A clean microscope slide was used to gently remove the perithecia with the ascospores from the surface of the agar. The perithecia/ascospore solution was placed in a sterile 50-mL tube and shaken vigorously 25 times. A hemocytometer was utilized to quantify the ascospore production of *C. globosum* isolates on the various $KClO_3$ treatments.

To further confirm the $KClO_3$ inhibitory activity on *C. globosum* growth and perithecia production, four other *C. globosum* isolates were grown on 0, 10, and 100 mM $KClO_3$-amended MEA. These experiments were repeated using CMC. Hyphal growth and perithecia were measured as described above for MEA treatments.

Experiments were conducted to determine the $KClO_3$ concentration that would significantly inhibit *C. globosum* isolates on MEA. Initial experiments using the *C. globosum* NM isolate utilized MEA plates amended with 0, 0.01, 0.1, 1, 10 mM $KClO_3$. This test was compared to similar concentrations applied to gypsum board. Similar experiments were repeated using MEA plates amended with 0, 0.1, 1. 10, 50, 100, and 200 mM of $KClO_3$ inoculated with three *C. globosum* isolates. Growth and perithecia were quantified as described previously.

Growth on Gypsum Board

Gypsum board was obtained from a local lumber yard. The gypsum board (wallboard/sheetrock) was cut into 5 cm × 5 cm pieces, placed in glass Petri dishes (90 mm diameter × 20 mm height), and autoclaved for 15 min at 121 psi. Once cooled, either sterile water or 1–10 mM $KClO_3$ was spread over the surface of the gypsum board. The solution was allowed to dry, and then 5×10^5 ascospores were distributed over the sterile sheetrock with a sterile glass rod. Sterile water (1 mL each week) was applied to the bottom of the Petri dish to maintain humidity. All inoculated media and glass Petri dishes were incubated in the dark at room temperature (25 °C). Perithecia were counted after 21 days of growth. Experiments were conducted 3 times with 3–9 replications per treatment.

Chaetomium globosum and Airborne Fungi Growth on Potassium Chlorate-amended Media

Chaetomium globosum (NM isolate) was grown on PDA for 3 weeks allowing abundant perithecia production. Sterile water (10 mL) was gently applied to the surface of the media. A sterile glass slide was used to gently dislodge perithecia/ascospores. The perithecia/ascospore suspension was filtered through a layer

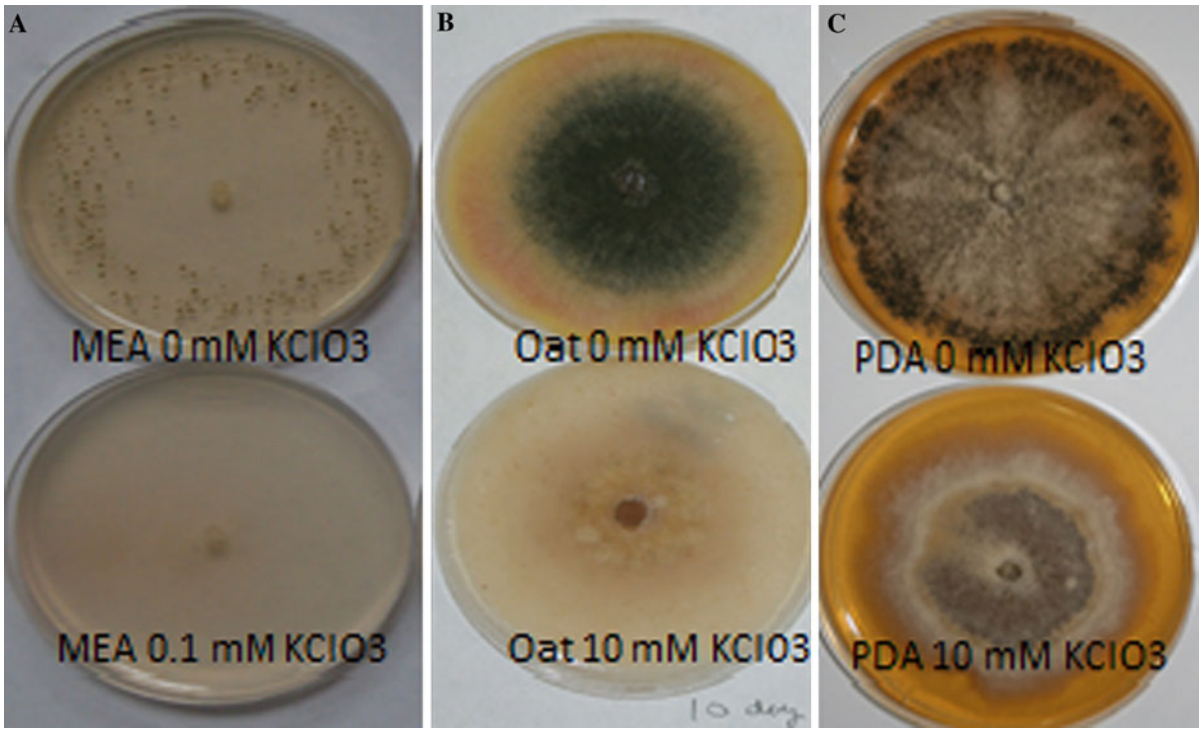

Fig. 2 Comparison of *C. globosum* perithecial growth on MEA, oat agar, and PDA amended with $KClO_3$. **a** MEA control with 0 mM $KClO_3$ and the *bottom plate* with 0.1 mM $KClO_3$ after 21 days of growth. **b** Oat agar control with 0 mM $KClO_3$ and the *bottom plate* with 10 mM $KClO_3$ after 10 days of growth. **c** Potato dextrose agar control with 0 mM $KClO_3$ and the *bottom plate* with 10 mM $KClO_3$ after 21 days of growth

of Miracloth™ (Calbiochem-Novabiochem Corp. La Jolla, CA) to separate the ascospores from the hypha and disrupted perithecia. Ascospores were quantified with a hemocytometer and then calibrated to 1,000 spores/mL. One hundred μL of the ascospores suspension was applied to MEA plates with various (1–100 mM) concentrations of $KClO_3$. The same technique was followed using the fungi *F. oxysporum*, *A. niger,* and *P. expansum*. These fungi were chosen because they are often found in water-damaged buildings. The spores were distributed over the media surface with a sterile glass rod. The plates were incubated in the dark at 25 °C, and colonies were counted after 72 h. The experiment was conducted two times with 4–5 replications per treatment.

In another experiment, MEA plates amended with $KClO_3$ (0–100 mM) were exposed to outside air utilizing an Aero-6™ sieve plate instrument (Aerotech laboratories, Inc. Phoenix, AZ). The instrument is very similar to an Andersen sampler and commonly used to quantify viable airborne fungal populations. The plates were placed in the sieve plate instrument, and 75 L of air was pumped through the device onto the $KClO_3$-amended plates for 3 min as recommended by the manufacturer. Plates were incubated in the dark at 25 °C for 72 h, and the fungal colonies counted. Each treatment was replicated 4–5 times, and the experiment was conducted 2 times.

Statistical Analysis

The data were analyzed using Graphpad Instat. Analysis of variance (ANOVA) and Tukey's comparison of means were conducted on the appropriate data sets emphasized in the results. Ascospore counts on the oat agar were also analyzed using Mann–Whitney paired test to compare the control with the $KClO_3$ treatments. Representative experiments were presented in the tables and figures. Each experiment described above was repeated at least 3 times unless otherwise stated, with 3–5 replicates per treatment.

Results

Growth and Perithecia Production on Various Media

After 6 days of growth on various media, the radial growth of *C. globosum* was greatest on oat and RM media when compared to the other media. *C. globosum* hyphae diameter after 6 days was 73 mm on oat and RM (Table 1). The rate of growth for *C. globosum* on CMC, CMA, MEA, QPDA, V8, PDA, and SNA was less (58–44 mm) than growth on oat and RM. CZ media showed the least growth of all the media tested. Development of perithecia was greatest on oat, then RM followed by V8, CMC, PDA, and MEA. Oat, RM, and PDA stimulated the most luxuriant hyphal growth (Fig. 2, oat and PDA). After 21 days, perithecia were observed in all media plates except CZ (Table 1).

Perithecia grown on MEA appeared on the surface of the media and at a density that could be accurately counted (Fig. 2a). Therefore, quantification of growth and perithecia synthesis was conducted using MEA as the standard media on most of the experiments conducted in this study.

Table 1 Hyphal growth and relative density of perithecia of *C. globosum* on various media

Media	Hyphal Growth after 6 days (mm)*	Perithecia after 21 days**
Oat	73a	+++
RM	73a	+++
CMC	58b	++
CMA	56b	+
MEA	56b	++
QPDA	55b	++
V8	48b	++
PDA	44b	++
SNA	44b	+
CZ	28c	–

Oat oat agar, *RM* rice agar, *CMC* 1 % carboxylmethyl cellulose agar, *CMA* cornmeal agar, *MEA* malt extract agar, *QPDA* quarter strength potato dextrose agar, *V8* V8 commercial juice amended with agar, *PDA* potato dextrose agar, *SNA* Spezieller Nährstoffarmer agar, *CZ* Csapek-Dox agar. All media were prepared according to standard laboratory techniques

* Numbers in the column followed by the same letter are not significantly different ($P < 0.05$) from each other according to Tukey–Kramer multiple comparisons test. Numbers followed by different letters are significantly different ($P < 0.05$) from one another

**, +++Very dense concentration of perithecia; ++ moderate density of perithecia; + low density of perithecia; –, No perithecia observed

Optimum Temperature Growth of *Chaetomium globosum*

Optimum growth and perithecial production for isolates appeared to be 25 and 30 °C (Table 2). The two *C. globosum* isolates found in leaf tissue (ECU 1490 and ECU SC-1) appeared to have a broader temperature range and grew well at 33 °C. The NMmt isolate was the slowest growing isolate at 25 and 30 °C. A significant ($P < 0.05$) reduction in growth was observed in most isolates at 33 °C. No growth occurred at 5 °C in any of the isolates. At 37 °C, minor hyphal growth was observed in 4 isolates, whereas 2 isolates (CB and ECU SC-1) indicated no growth. Perithecia were observed at 25 °C in all isolates except CB after 19 days of incubation, and all isolates produced perithecia at 30 °C after 19 days. Whereas hyphal growth was significantly reduced in most isolates incubated at 33 °C, 5 of the 6 isolates (NMmt did not) produced perithecia after 19-day incubation.

When comparing growth on PDA and MEA at 25 and 30 °C, there was no significant difference in growth after 14 days among *C. globosum* isolates (Table 3). Perithecia production was observed in all isolates grown on MEA after 14 days incubated at 25 and 30 °C. Only one isolate (ECU SC-1) produced observable perithecia at 30 °C on PDA after 14-day inoculation. Based upon the information collected in Tables 2 and 3, the $KClO_3$-amended media experiments were conducted at 25 °C and MEA was the standard media for most experiments.

Growth and Perithecia Synthesis of *Chaetomium globosum* on Solid Media Amended with $KClO_3$

Chaetomium globosum hyphal growth after 7 days in 2 g/L (16 mM) amended MEA was significantly different from the control (Table 4). All $KClO_3$ concentrations (2–15 g/L; 41–122 mM) reduced hyphae growth approximately 6 % after 7 days of growth. Growth of *C. globosum* in broth culture was reduced approximately 30 % within a concentration range of 16–122 mM (Fig. 3). Perithecia were counted after 21 days of growth. The lowest concentration of $KClO_3$ reduced perithecia formation, and no

Table 2 Growth of *C. globosum* isolates at various temperatures on potato dextrose agar (PDA) and the incidence of perithecia after 14 days of incubation

Isolates	Temperature (°C)	Fungal diameter after 14 days(mm)	Perithecia incidence
NM	5	0[d]*	−**
ECU 1490	5	0[d]	−
ECU SC-1	5	0[d]	−
NMmt	5	0[d]	−
PI-932	5	0[d]	−
CB	5	0[d]	−
NM	25	60[a]	+
ECU 1490	25	71[a]	+
ECU SC-1	25	61[a]	+
NMmt	25	55[b]	+
PI-932	25	68[a]	+
CB	25	65[a]	−
NM	30	73[a]	+
ECU 1490	30	76[a]	+
ECU SC-1	30	71[a]	+
NMmt	30	62[b]	+
PI-932	30	82[a]	+
CB	30	72[a]	+
NM	33	30[c]	+
ECU 1490	33	59[a]	+
ECU SC-1	33	48[a]	+
NMmt	33	35[c]	−
PI-932	33	35[c]	+
CB	33	51[b]	+
NM	37	3[d]	−
ECU 1490	37	5[d]	−
ECU SC-1	37	0[d]	−
NMmt	37	7[d]	−
PI-932	37	6[d]	−
CB	37	0[d]	−

* Numbers in the column followed by the same letter are not significantly different ($P < 0.05$) from one another according to Tukey–Kramer multiple comparisons test

** A "−" mark indicates that perithecia were not present on any of the replications after 19 days. A "+" indicates that perithecia were present on one of more of the replications after 19 days

perithecia were observed on the media in the higher $KClO_3$ concentrations (Table 4, Fig. 2). In further experiments to determine whether lower levels of $KClO_3$ could inhibit perithecia synthesis, perithecia

Table 3 A comparison of growth of *C. globosum* isolates at 25 and 30 °C on either PDA or MEA and the incidence of perithecia after 14 days of incubation

Isolates	Temperature (°C)	Fungal diameter after 14 days(mm)*		Perithecia incidence**	
		PDA	MEA	PDA	MEA
NM	25	66[b]	85[a]	−	+
ECU 1490	25	68[b]	85[a]	−	+
ECU SC1	25	60[b]	85[a]	−	+
NMmt	25	62[b]	85[a]	−	+
CB	25	60[b]	85[a]	−	+
NM	30	59[b]	85[a]	−	+
ECU 1490	30	68[b]	81[a]	−	+
ECU SC1	30	62[b]	77[a]	+	+
NMmt	30	52[b]	85[a]	−	+
CB	30	59[b]	85[a]	−	+

* Numbers in the two fungal diameter columns followed by the same letter are not significantly different ($P < 0.05$) from one another according to Tukey–Kramer multiple comparisons test

** A "−" mark indicates that perithecia were not present on any of the replications after 14 days. A "+" indicates that perithecia were present on one of more of the replications after 14 days

synthesis was inhibited at 0.01 mM and no perithecia grew on the media with 1 mM $KClO_3$ (Fig. 4). A similar experiment was conducted with 3 *C. globosum* isolates in which the $KClO_3$ range was 0.1–200 mM. All three isolates showed a 10–30 % reduction in hyphal diameter among the 0.1–100 mM $KClO_3$ treatments (Table 5). Perithecial production was reduced almost completely at 0.1 mM $KClO_3$, and no perithecia were observed on plates amended with 10–200 mM $KClO_3$. Hyphal growth was inhibited at 200 mM when compared to the 0.1–100 mM $KClO_3$ treatments in the NM and ECU-lib isolates. When comparing perithecia on MEA and gypsum board, a 10× higher concentration of $KClO_3$ was needed to inhibit perithecia on gypsum board (Fig. 4).

When comparing the $KClO_3$ inhibition of *C. globosum* (NM isolate) grown on MEA and oat agar, a similar trend was observed (Table 4). However, oat agar produced a profusion of perithecia compared to MEA and PDA (Fig. 2). Therefore, ascospores produced per plate were determined using a hemocytometer. Assuming that the same number of ascospores is produced in each perithecium, the number of ascospores should reflect the relative number of perithecia.

Table 4 Hyphal growth, perithecia, and ascospore synthesis of *C. globosum* (NM isolate) on MEA and oat agar amended with various concentrations of potassium chlorate

Media	[KClO3] (mM)	Hyphal growth (mm) Day 7[a]	Perithecia/ Plate Day 21	Ascospores/ Plate (X 10^4) Day 14
MEA	0	51	51	–
	16	48*	3*	–
	41	47*	0*	–
	57	49*	0*	–
	81	49*	0*	–
	122	48	0*	–
Oat agar	0	71	TNTC[b]	40,375[a,c]
	1	64*	–	389*
	10	64*	–	1,070*
	100	71	–	275*

[a] Numbers in the individual columns with a * are significantly different ($P < 0.05$) from the 0 mM control according to the Tukey–Kramer multiple comparisons test

[b] TNTC, too numerous to count. Ascospores from the Petri dishes were compared among treatments instead of perithecia

[c] The Mann-Whitney paired test indicated a significant difference between the oat 0 mM control and the combined KClO3 (1, 10, 100 mM) data ($P = 0.0005$)

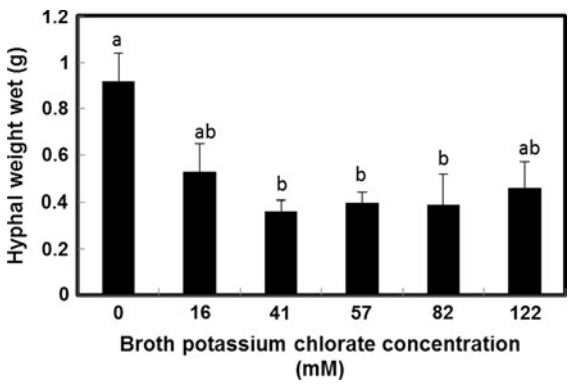

Fig. 3 MEA broth amended with potassium chlorate ($KClO_3$) and inoculated with *C. globosum* hyphal disks. *Vertical lines* indicate standard error of the mean. *Columns with different letters* are significantly different from one another ($P < 0.05$) according to the Tukey–Kramer multiple comparisons test

Ascospores harvested from the control 0 mM oat agar plates were significantly higher (38–146 times) than the $KClO_3$ treatments. There was no significant difference between the $KClO_3$ treatments.

Experiments were conducted to determine whether the $KClO_3$ inhibition was unique to the NM isolate. Six *C. globosum* isolates were grown on $KClO_3$ MEA-amended media (Table 6). All isolates showed a similar trend in hyphal growth and perithecia production as the NM isolate. The NMmt isolate produced the highest number of perithecia and the CB isolate produced the least number of perithecia when comparing controls for each isolate (0 mM $KClO_3$ treatment).

An additional media experiment was conducted to determine whether CMC, a highly cellulytic media, would interact with $KClO_3$ in the same manner as MEA and oat agar (Table 7). Four isolates were compared on 0, 10, and 100 mM $KClO_3$. CMC agar stimulated a higher number of perithecia when compared to MEA (Tables 4,5,6). The same trend in the significant perithecia reduction on $KClO_3$-amended CMC was observed on $KClO_3$-amended MEA and oat agar.

Growth of *Chaetomium globosum* in Broth Culture

Growth of *C. globosum* in MEA broth was significantly greater in the control (MEA broth only) and 2 g/L (16 mM) $KClO_3$-amended MEA broth, when compared to growth in MEA amended with 5–10 g/L (41–82 mM) $KClO_3$ (Fig. 3). There was no significant difference in the hyphal weight of *C. globosum* grown in the higher concentrations of $KClO_3$. These results indicate that $KClO_3$ does not completely inhibit hyphal growth of *C. globosum* in MEA broth at relatively high concentrations (122 mM). This same trend was observed when *C. globosum* was grown on solid media (Tables 4, 5, 6 and 7 and Figs. 2, 4).

In summary, the experiments represented in Tables 4, 5, 6, and 7 and Figs. 2, 3, and 4 indicate that low levels of $KClO_3$ inhibit *C. globosum* perithecia production on MEA, oat agar, CMC, and gypsum board. The reduction in hyphal growth and weight was not completely inhibited at the highest levels of $KClO_3$ tested.

Germination of *Chaetomium globosum* Ascospores, Other Indoor Molds, and Airborne Fungal Spores on Media Amended with Various Concentrations of Potassium Chlorate

Germination of *C. globosum* ascospores, *F. oxysporum* conidiospores (primarily macroconidia), *A. niger* conidia, and *P. expansum* conidia was not inhibited

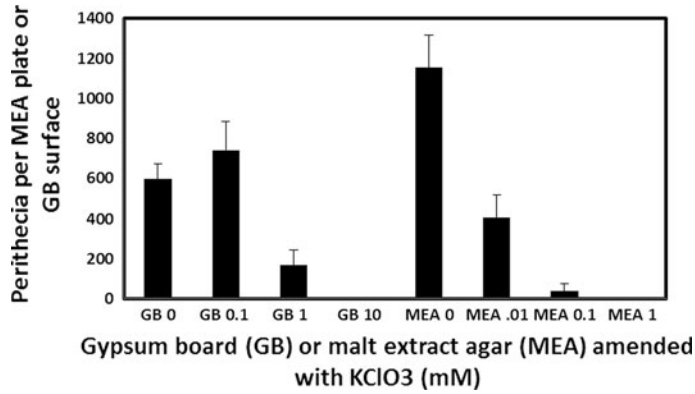

Fig. 4 *Chaetomium globosum* perithecia growth on MEA and gypsum board (GB) with different concentrations of $KClO_3$. *Vertical lines* indicate standard error of the mean

Table 5 Comparison of concentration levels of $KClO_3$-amended MEA on growth and perithecia production of *Chaetomium* isolates

Isolate	[$KClO_3$] (mM)	Fungal diameter (7 d)[A]	Perithecia/plate (21 d)[B]
NM	0	58[a]	406
	0.1	49[b]	2*
	1	46[b]	0*
	10	48[b]	0*
	50	52[b]	0*
	100	50[b]	0*
	200	35[c]	0*
ECU-lib	0	60[a]	827
	0.1	48[d]	55*
	1	50 [cd]	1*
	10	48[d]	0*
	50	52[bc]	0*
	100	52[b]	0*
	200	40[e]	0*
PI-932	0	56[a]	839
	0.1	40[c]	0*
	1	39[c]	0*
	10	40[c]	0*
	50	42[c]	0*
	100	46[b]	0*
	200	47[b]	0*

[A] The statistical analysis did not determine variation among isolates only within an isolate. Numbers in the individual columns and individual isolates with a different letter are significantly different ($P < 0.05$) from one another according to the Tukey–Kramer multiple comparisons test

[B] Numbers in the individual columns with an * are significantly different ($P < 0.05$) from the 0 mM control according to the Tukey–Kramer multiple comparisons test

Table 6 The comparison of growth and perithecia production of *C. globosum* isolates when grown on MEA amended with different concentrations of potassium chlorate ($KClO_3$)

Isolates	[$KClO_3$] (mM)	Fungal diameter (7 d)*	Perithecia/plate (21 d)**
NM	0	47[b]	115[b]
	10	37[c]	1[de]
	100	45[b]	0[de]
NMmt	0	50[b]	224[a]
	10	47[b]	2[de]
	100	52[b]	0[de]
PI 932	0	50[ab]	50 [cd]
	10	40[c]	1[e]
	100	49[b]	0[e]
CB	0	49[a]	16[e]
	10	38[b]	6[e]
	100	44[b]	0[e]
ECU 1490	0	55[a]	80[c]
	10	47[b]	25[de]
	100	49[b]	0[e]
ECU S-1C	0	55[a]	122[b]
	10	47[b]	0[de]
	100	48[b]	1[de]

* The statistical analysis did determine variation among the isolates and in regard to hyphal growth and perithecia synthesis. Numbers in each column followed by the same letter are not significantly different ($P < 0.05$) from one another

** Perithecia per plate, four plates per treatment

when exposed to 0–100 mM $KClO_3$ (Table 8). The number of fungal colonies expressed as colony-forming units was not significantly different when

Table 7 The comparison of growth and perithecia production of *C. globosum* isolates grown on CMC amended with different concentrations of potassium chlorate ($KClO_3$)

Isolates	[$KClO_3$] (mM)	Fungal diameter (7 d)*	Perithecia/plate (21 d) **
NM	0	70^{bc}	1288^{b}
	10	66^{c}	184^{c}
	100	74^{b}	86^{c}
NMmt	0	62^{c}	2651^{a}
	10	71^{b}	358^{c}
	100	85^{a}	297^{c}
PI 932	0	66^{bc}	1010^{b}
	10	59^{d}	84^{c}
	100	68^{bc}	59^{c}
CB	0	62^{d}	1676^{b}
	10	60^{d}	304^{c}
	100	71^{b}	223^{c}

* The statistical analysis did determine variation among the isolates and in regard to hyphal growth and perithecia synthesis. Numbers in each column followed by the same letter are not significantly different ($P < 0.05$) from one another

** Perithecia per plate, four plates per treatment

comparing the treatment means within the fungal species tested.

MEA plates amended with 0–100 mM were exposed to outdoor air utilizing an Aero-6 sieve plate instrument. The predominant genera appeared to be dematiaceous species. The number of colony-forming units was not significantly different when comparing the means within the different levels of $KClO_3$ tested. These results suggest that $KClO_3$ does not inhibit fungal spore germination on a number of fungal species. However, this aspect needs to be further investigated with more detailed identification.

Discussion

Growth of *C. globosum* (NM isolate) was significantly ($P < 0.05$) greater on RM and oat when compared to the other eight media tested. Andersen and Nissen [3] showed that media containing cereals and vegetables stimulated greater growth and increased sporulation of *Stachybotrys* and *Chaetomium* isolates. They found consistent growth but variable sporulation on CZ, MEA, and V8 and several other media not tested in this study. The slowest rate of growth in the present study was observed on CZ, which is consistent with the results of Anderson and Nissen [3]. However, we observed consistent perithecia production on MEA (lower concentration of malt extract than normal formula) and V8 agar. MEA was chosen as the media in our experiments because of the quantifiable perithecia production compared to the luxuriant perithecia growing on oat and PDA (Table 1, Fig. 2). MEA enabled the observer to more accurately quantify perithecia production on the media plates. Optimum temperature for *C. globosum* growth and perithecia

Table 8 Spore germination (CFU, colony-forming units) after 72-h incubation of *C. globosum*, *A. niger*, *F. oxysporum*, *P. expansum,* and airborne fungi on MEA with various levels of potassium chlorate

[$KClO_3$ mM]	*C. globosum*	Germination of spores (CFU) on $KClO_3$-amended media*			
		Aspergillus	*Fusarium*	*Penicillium*	Sieve plate test**
0	152 ± 17	134 ± 9.3	207 ± 19	306 ± 37	328 ± 11
0.01	154 ± 17	–	–	–	–
0.1	152 ± 24	–	–	–	–
1.0	127 ± 18	88 ± 11	286 ± 43	387 ± 62	334 ± 26
10	135 ± 21	115 ± 19	280 ± 67	382 ± 66	306 ± 17
50	104 ± 10	–	–	–	–
100	205 ± 38	108 ± 23	209 ± 27	366 ± 52	334 ± 22

* The numbers within each column represent the mean and the standard error of the mean. According to Tukey–Kramer multiple comparisons test, there were no significant differences between the means in the same column

** Outdoor air was forced through a Aero-6^{TM} sieve plate instrument onto a Petri dish containing various levels of potassium chlorate ($KClO_3$)

CFU, colony-forming units

production was found to be approximately 25–30 °C for the six isolates tested and corresponds with the fungal growth temperatures used by others [3, 15, 16, 39].

Mycelial growth in broth cultures was not significantly inhibited until $KClO_3$ concentrations were greater than 41 mM (Fig. 3). Increasing $KClO_3$ concentrations tenfold did not significantly reduce hyphal weight, suggesting that *C. globosum* has a high tolerance for $KClO_3$. Mycelial growth of three *C. globosum* isolates on solid media (MEA) was inhibited at much lower $KClO_3$ concentrations (0.1 mM). However, there was no significant difference in growth ($P < 0.05$) among the 0.1 and 100 mM $KClO_3$ concentrations. *C. globosum* growth rates in broth culture and solid media amended with various $KClO_3$ concentrations align closely to a logarithmic growth curve ($R^2 = 0.93$).

In contrast to hyphal growth, perithecia production on solid media was significantly reduced when *C. globosum* was grown on MEA amended with 0.01 mM $KClO_3$, and no perithecia production was observed at 1 mM $KClO_3$. In order to test these effects on building materials, $KClO_3$ liquid suspensions were applied to gypsum board. Applications of 1 mM $KClO_3$ on gypsum board reduced perithecia production and 10 mM completely inhibited perithecia growth (Fig. 4). When oat agar was amended with $KClO_3$, a higher number of perithecia were observed compared to MEA and CMC. In order to quantify the difference between oat agar treatments, ascospores were harvested from the plates and counted. A significant reduction in ascospore synthesis was observed between the 0 mM control and the $KClO_3$ treatments (Table 4). Qualitative differences in C. *globosum* perithecia were also observed on PDA amended with $KClO_3$ (Fig. 2). However, perithecia were too numerous to count on PDA, and harvesting of ascospores was not successful.

The possibility that $KClO_3$ inhibited ascospore germination rather than perithecia production was tested. *C. globosum* ascospores were spread over MEA plates and observed for germination and quantified by counting *C. globosum* colonies. There was no significant reduction in ascospore germination when compared to controls. When further experiments were conducted by exposing media plates amended with 0–100 mM $KClO_3$ to other fungal species often found growing on indoor building materials (Table 8) or to fungal spores collected from outdoor air, the $KClO_3$-amended media (1–100 mM) did not inhibit germination of the other four species tested or the airborne fungal spores. According to these results, it appears that spores of *C. globosum* and several other fungi are not inhibited by $KClO_3$.

Chaetomium globosum is highly resistant to many environmental stresses. *C. globosum* exhibits tolerance to salts [6] and has the ability to recolonize and grow on building materials exposed to high levels of radiation [44]. Wilson et al. [41] showed that chlorine dioxide completely inhibited *Stachybotrys chartatum*, *Penicillium chrysogenum*, and *Cladosporium cladosporium*, whereas, 11 % of *C. globosum* ascospores were not inactivated by the gas. The *C. globosum* perithecia were implicated as a protective factor against chlorine dioxide infiltration. *C. globosum* was highly tolerant of LiCl when compared to six other ascomycete fungi [34] and several other imperfect fungi. As the results of this paper indicate, *C. globosum* appears to be tolerant to relatively high levels of $KClO_3$ in regard to hyphal growth and spore germination.

$KClO_3$ toxicity has been observed in several species including algae, bacteria, and fungi. Toxicity has been associated with the metabolism of chlorate. Nitrate reductase normally reduces nitrate, but can also reduce chlorate to the more toxic chlorite [18, 39]. Strains of *Ustilago maydis* and *Escherichia coli* that lack nitrate reductase were found to be resistant to chlorate [28, 30], whereas wild types were chlorate-sensitive. Another toxicity pathway may involve chlorite dismutase that reduces perchlorate to chloride, resulting in a disproportionate amount of chlorite [35]. Cove [9], working with *A. nidulans*, proposed that chlorate toxicity was associated with the activity of nitrate reductase and the product of the nirA gene. NirA is a up-regulator for nitrate assimilation. Cove [9] also noted that various nitrogen sources affect chlorate sensitivity and that *A. nidulans* grown in the presence of ammonium reduces nitrogen catabolic activity. Low pH induces toxicity in *A. nidulans* in a pattern similar to chlorate. However, chlorate up to 500 mM did not affect the pH of the basal media. Fogel [16] observed *C. globosum* perithecia and ascospores when grown on media with pH of 6–8.6. Sporulation was best in an acidic environment. Our study showed no major change in pH due to addition of $KClO_3$ to the broth or agar media and abundant sporulation at pH 5.5–6. Our study did not indicate that

pH played a role in the inhibition of *C. globosum* perithecia synthesis.

$KClO_3$ has been used to stimulate heterokaryons in several fungi, but most notably in *F. oxysporum* [8, 31]. When *F. oxysporum* was grown in 15 g/L of $KClO_3$, 3 distinct phenotypes were classified that presumably were the result of mutations in nitrate reductase (Nit1), nitrate assimilation pathway-specific regulatory locus (Nit3), and molybdenum-cofactor of nitrate reductase (NitM). The presence of nitrate reductase in *C. globosu*m is not known. The present study did indicate that *C. globosum* hyphal growth was highly resistant to $KClO_3$. *Trichoderma hamatum* was considered highly resistant to chlorate at $\geq$7.48 mM [39], whereas *C. globosum* hyphal growth on 10 mM $KClO_3$ was not significantly less than the control after 4 days of growth, and was never reduced more than 30 % up to 200 mM $KClO_3$. Our results show that many fungi appear to be resistant to chlorate toxicity. Hyphal growth in broth culture showed a similar trend to that observed on agar plates. In contrast, perithecia were inhibited by very low $KClO_3$ levels (0.01 mM $KClO_3$). Johnson [25] predicted that 200–300 genes are involved in perithecia formation. At this time, the mechanism of perithecia inhibition and the perithecia-forming genes that may be mutated by $KClO_3$ are not known.

As indicated above, *C. globosum* is highly resistant to many environmental stresses. The differential response of hyphal growth and perithecia formation to $KClO_3$ needs further investigation to elucidate the genes involved in reproduction of *C. globosum*. Further research in the formulation and application of $KClO_3$ to building materials may prove to be an efficient method of inhibiting growth of fungi like *C. globosum*. Our laboratory is currently investigating the *C. globosum* proteins expressed when grown in low $KClO_3$ concentrations (0.1–1 mM). The identification of these proteins will lead to genes that may be involved in perithecia inhibition. In additional, further experiments are being conducted on the effect of the type and amount of nitrogen on *C. globosum* growth and reproduction. This information will help to improve our basic understanding of perithecia formation and, perhaps, provide information that will contribute to the control of fungal growth in water-damaged buildings.

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References

1. Alexopoulos CJ, Mims CW, Blackwell M. Introductory mycology. 4th ed. London: Wiley; 1996.
2. Anandi V, John TJ, Walter A, Shastry JCM, Lalitha MK, Padhye AA, Ajello L, Chandler FW. Cerebral phaeohyphomycosis caused by *Chaetomium globosum* in a renal transplant recipient. J Clin Micro. 1989;27:2226–9.
3. Andersen B, Nissen AT. Evaluation of media for detection of *Stachybotrys* and *Chaetomium* species associated with water-damaged buildings. Int Biodeterior Biodegradation. 2000;46:111–6.
4. Andersson MA, Nikulin M, Köljalg U, Andersson MC, Rainey F, Reijula K, Hintikka E-L, Salkinoja-Salonen M. Bacteria, molds, and toxins in water-damaged building materials. Appl Environ Microbiol. 1997;63:387–93.
5. Bush RK, Portnoy JM, Saxon A, Terr AI, Wood RA. The medical effects of mold exposure. J Allergy Clin Immunol. 2006;117:326–33.
6. Cantrell SA, Casillas-Martínez L, Molina M. Characterization of fungi from hypersaline environments of solar slatterns using morphological and molecular techniques. Mycol Res. 2006;110:962–70.
7. Chiu A, Fink JN. Fungal allergy and pathogenicity. Introduction. Chem Immunol. 2002;81:1–4.
8. Correll JC, Klittich CJR, Leslie JF. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology. 1987;77:1640–6.
9. Cove DJ. Chlorate toxicity in *Aspergillus nidulans*. Mol Gen Genet. 1976;146:147–59.
10. Dhingra OD, Mizubuti ESG, Santana FM. *Chaetomium globosum* for reducing primary inoculum of *Diaporthe phaseolorum* f. sp. *meridionalis* in soil-surface soybean stubble in field conditions. Biol Control. 2003;26:302–10.
11. Di Pietro A, Gut-Rella M, Pachlatko JP, Schwinn FJ. Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum,* a causal agent of damping-off. Phytopathology. 1992;82:131–5.
12. Dunn-Coleman NS, Smarrelli J, Garrett RH. Nitrate assimilation in eukaryotic cells. Int Rev Cytol. 1984;92:1–50.
13. Edmundson DA, Nordness ME, Zacharisen MC, Kurup VP, Fink JN. Allergy and "toxic mold syndrome". Ann Allergy Asthma Immunol. 2005;94:234–9.
14. Febre N, Silva V, Medeiros EAS, Godoy P, Reyes E, Halker E, Fischman O. Contamination of peritoneal dialysis fluid by filamentous fungi. Rev Iberoam Micol. 1999;16:238–9.
15. Fogel MR, Douglas DR, Jumper CA, Straus DC. Growth and mycotoxin production by *Chaetomium globosum*. Mycopathologia. 2007;164:49–56.
16. Fogel MR, Douglas DR, Jumper CA, Straus DC. Growth and mycotoxin production by *Chaetomium globosum* is favored in a neutral pH. Int J Mol Sci. 2008;9:2357–65.
17. Genuis SJ. Clinical medicine and the budding science of indoor mold exposure. Eur J Int Med. 2007;18:516–23.

18. Goksøyr J. On the effect of chlorate upon the nitrate reduction of plants. I. Experiments with *Aspergillus oryzae*. Physiol Plant. 1951;4:498–513.
19. Guarro J, Soler L, Rinaldi MG. Pathogenicity and antifungal susceptibility of *Chaetomium* species. Eur J Clin Microbiol Infect Dis. 1995;14:613–8.
20. Hanlin RT. Illustrated genera of the acomycetes, vol. I. St. Paul: APS Press; 1990.
21. Hattori N, Adachi M, Kaneko T, Shimozuma M, Ichinohe M, Iozumi K. Case report. Onychomycosis due to *Chaetomium globosum* successfully treated with itraconazole. Mycoses. 2000;43:89–92.
22. Horner WE, Worthan AG, Morey PR. Air-and dustborne mycoflora in houses free of water damage and fungal growth. Appl Environ Microbiol. 2004;70:6394–400.
23. Jarvis BB, Miller JD. Mycotoxins as harmful indoor air contaminants. Appl Microbiol Biotechnol. 2005;66: 367–72.
24. Johanning E. Indoor moisture and mold-related health problems. Allerg Immunol. 2004;36:182–5.
25. Johnston TE. Isolation and characterization of perithecial development mutants in *Neurospora*. Genetics. 1978;88: 27–47.
26. Köhl J, Bélanger RR, Fokkema NJ. Interaction of four antagonistic fungi with *Botrytis aclada* in dead onion leaves: a comparative microscopic and ultrastructural study. Phytopathology. 1997;87:634–42.
27. Korpi A, Pasaneen A, Pasanen P. Volatile compounds originating from mixed microbial cultures on building material under various humidity conditions. Appl Environ Microbiol. 1998;64:2914–9.
28. Lewis CM, Fincham JRS. Genetics of nitrate reductase in *Ustilago maydis*. Genetic Res Camb. 1970;16:151–63.
29. Ohtsubo K, Saito M, Sekito S, Yoshihira K, Natori S. Acute toxic effects of chaetoglobosin A, a new cytochalasan compound produced by *Chaetomium globosum*. Jpn J Exp Med. 1978;48:105–10.
30. Piéchard M, Puig J, Pichinoty F, Azoulay E, Le Minor L. Mutation affectant la nitrate-reductase A et d'autre enzymes bactreriennes d'oxydo-reduction. Etude preliminaire. Ann Inst Pasteur. 1967;112:24–37.
31. Puhalla JE. A visual indicator of heterokaryosis in *Fusarium oxysporum* from celery. Can J Bot. 1984;62:540–5.
32. Rakotonirainy MS, Heude E, Lavédrine B. Isolation and attempts of biomolecular characterization of fungal strains associated to foxing on the 19th century book. J Cultural Herit. 2007;8:126–33.
33. Rao CY, Riggs MA, Chew GL, Muilenberg ML, Thorne PS, Sickle DV, Dunn KH, Brown C. Characterization of airborne molds, endotoxins, and glucans in homes in New Orleans after hurricanes Katrina and Rita. Appl Environ Microbiol. 2007;73:1630–4.
34. Richter DL, Robinson SC, Beardslee MP, Habarth ML. Differential sensitivity of fungi to lithium chloride in culture media. Mycol Res. 2008;112:717–24.
35. Rikken GB, Kroon AGM, van Ginkel CG. Transformation of (per)chlorate into chloride by a newly isolated bacterium: reduction and dismutation. Appl Microbiol Biotechnol. 1996;45:420–6.
36. Rippon JW. Medical mycology. The pathogenic fungi and pathogenic actinomycetes. 3rd ed. Philadelphia: The W.B. Saunders Co.; 1998.
37. Szczepanowska M, Moomaw WR. Laser stain removal of fungus-induced stains from paper. JAIC. 1994;33:25–32.
38. Tullio V, Bance G, Allizond B, Roana J, Mandras N, Scalas D, Panzone M, Cervetti O, Valle S, Carlone N, Cuffini AM. Non-dermatophyte moulds as skin and nail foot mycosis agents: *Phoma herbarum, Chaetomium globosum* and *Microascus cinereus*. Fungal Biol. 2010;114:345–9.
39. Van Wijk DJ, Kroon GM, Garttener-Avends CM. Toxicity of chlorate and chlorite to selected species of algae, bacteria and fungi. Ecotoxicol Environ Saf. 1998;40:206–11.
40. Wijeratne EMK, Turbyville TJ, Fritz A, Whitesell L, Gunatilaka AAL. A new dihydroxanthenone from a plant-associated strain of the fungus *Chaetomium globosum* demonstrates anticancer activity. Bioorg Med Chem. 2006;14:7917–23.
41. Wilson SC, Wu C, Andriychuk LA, Martin JM, Brasel TL, Jumper CA, Straus DC. Effect of chlorine dioxide gas on fungi and mycotoxins associated with Sick Building Syndrome. Appl Environ Microbiol. 2005;71:5399–403.
42. Wright D, Biles C. Environmental and potassium chlorate effects on the growth of *Chaetomium globosum*. Proc Oklahoma Acad Sci. 2006;86:103.
43. Young J, Fuego M, Pattison R, Biles CL. Growth and reproduction of *Chaetomium globosum* on various carbon sources exposed to different light regimes. Proc Oklahoma Acad Sci. 2008;88:53.
44. Zhdanova NN, Zakharchenko A, Vember VV. Fungi from Chernobyl: mycobiota of the inner regions of the containment structure of the damaged nuclear reactor. Mycol Res. 2000;104:1421–6.