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Antifungal properties of silver nanoparticles against indoor mould growth

Anna Ogar [a,*], Grzegorz Tylko [b], Katarzyna Turnau [a,c]

[a] Plant-Microbial Interaction Research Group, Institute of Environmental Science, Jagiellonian University, Krakow, Poland
[b] Department of Cell Biology and Imaging, Institute of Zoology, Jagiellonian University, Krakow, Poland
[c] The Malopolska Center of Biotechnology, Jagiellonian University, Krakow, Poland

HIGHLIGHTS

- Growth of common indoor fungi is inhibited in the presence of silver nanoparticles.
- Silver nanoparticles cause morphological changes in fungi.
- Fungi reproductive structures are affected by silver nanoparticles.
- Silver nanoparticles might stimulate growth of fungal species.

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ABSTRACT

The presence of moulds in indoor environments causes serious diseases and acute or chronic toxicological syndromes. In order to inhibit or prevent the growth of microorganisms on building materials, the disruption of their vital processes or the reduction of reproduction is required. The development of novel techniques that impair the growth of microorganisms on building materials is usually based on silver nanoparticles (AgNPs). It makes them an alternative to other biocides. AgNPs have proven antibacterial activity and became promising in relation to fungi. The aim of the study was to assess growth and morphology of mycelia of typical indoor fungal species: *Penicillium brevicompactum*, *Aspergillus fumigatus*, *Cladosporium cladosporoides*, *Chaetomium globosum* and *Stachybotrys chartarum* as well as *Mortierella alpina*, cultured on agar media. The antifungal activity of AgNPs was also tested in relation to *C. globosum* and *S. chartarum* grown on the surface of gypsum drywall. It was found that the presence of AgNPs in concentrations of 30–200 mg/l significantly decreased the growth of fungi. However, in the case of *M. alpina*, AgNPs stimulated its growth. Moreover, strong changes in moulds morphology and colour were observed after administration of AgNPs. Parameters of conidiophores/sporangiophores varied depending on mould region and changed significantly after treatment with AgNPs. The experiments have shown antifungal properties of AgNPs against common indoor mould species. Their application to building materials could effectively protect indoor environments from mould development. However, consideration must be given to the fact that the growth of some fungal strains might be stimulated by AgNPs.

1. Introduction

Present-day urban society spends approximately 85–90% of its time inside buildings. A strong trend in modern construction is energy saving; thus, the demand for new technologies to significantly reduce heat exchange between interior parts of buildings and the exterior environment (Chamakura et al., 2011). Current building materials, when exposed to high relative humidity in areas of poor or inappropriate lightning, heating or ventilation, are frequently found to be excellent substrates for mould formation. This leads to indoor air quality problems and is directly associated with "sick building syndrome" (SBS) and "building related illness" (BRI). People who reside in fungi affected indoor environments might experience their health getting worse and/or feel discomfort after spending long amounts of time indoors (The Environmental Protection Agency — EPA). The SBS has been linked mostly to the presence of allergens, antigens, β-1,3-glucans, mycotoxins and microbial volatile organic compounds (MVOCs) (Crook and Burton, 2010; Shoemaker and House, 2005; Tuomi et al., 2000). Due to prolonged exposure of human organism to these factors, the respiratory, circulatory, and nervous systems are affected. Other negative impacts are skin irritation and non-specific hypersensitivity reactions and the development of cancer (Shoemaker and House, 2005).

* Corresponding author.
E-mail addresses: anna.ogar@uj.edu.pl (A. Ogar), grzegorz.tylko@uj.edu.pl (G. Tylko), katarzyna.turnau@uj.edu.pl (K. Turnau).

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Aspergillus fumigatus is the most common fungal species that occupies indoor environments and causes allergic bronchi-pulmonary aspergillosis. This disease leads to the fatal destruction of lung tissue (Latge, 1999; Bush and Portnoy, 2001). Another species, *Penicillium brevicompactum*, produces chemical compounds like mycophenolic acid, asperphenamate, brevianamide A, and roquefortine C, a tanzawaid acid analogue that was reported to be strong allergens. They may cause hypersensitivity pneumonitis and allergic alveolitis, especially in susceptible individuals. Moreover, other infections such as keratitis, penicilliosis, and otomycosis were noted (Bush and Portnoy, 2001; Cooley et al., 2004; Shen et al., 1996). Mycelium of *Cladosporium cladosporoides* that commonly grows on damp indoor surfaces and causes allergic reactions or asthma symptoms due to the production of mycotoxins such as asperentin, cladosporic acid, sterigmatocystin and diacetoxyscirpenol (DAS) (Bush and Portnoy, 2001; Tuomi et al., 2000). Residents of buildings highly affected with *Stachybotrys chartarum* manifested adverse health effects due to the many bioactive compounds released by these types of moulds: macrocylic trichothecenes, related trichoverroids (Andersen et al., 2002; Bennett and Klich, 2003; Kuhn and Ghannoum, 2003), phenylspirodrimanes (spirolactones and spirolactams) and cyclosporins (potent immunosuppressive agents) (Nielsen et al., 2002; Nielsen, 2003). Some of these metabolites could cause respiratory, dermatological, eye and constitutional symptoms (Gravesen et al., 1994), or they have suppressant effects on the immune system Jarvis and Miller (2005). Over 40 chaetoglobosins were isolated and characterized from the *Chaetomium globosum* species present on the paper (cellulose) surface of gypsum boards. Many of them demonstrated acute toxicity to mammals and strong cytotoxicity to cells. *C. globosum* produces two mycotoxins (chaetoglobosins A and C) that belong to the group of cytochalasins, known as actin binding compounds (Bloch, 1973; Fogle et al., 2007; Griffin et al., 1982).

Mortierellales constitutes one of the largest groups of zygomycetes. Most of the *Mortierella* species are known as soil born fungi, while we have been able to isolate *Mortierella alpina* from basements of the Cracovian Market Square. *M. alpina* has biotechnological significance as the producer of polyunsaturated fatty acids (PUFA); it might contain more than 25% of lipids in its biomass. *Mortierella isabellina* has been applied in various regio- and stereospecific biotransformations. *Mortierella wolfii* is reported as an animal pathogen causing bovine mycotic abortion, pneumonia and systemic mycosis (Certik and Shimizu, 1999; Domsch et al., 1980; Dyal and Narine, 2005; Jefferys et al., 1953). Information provided by the Occupational Safety & Health Administration of the United States Department of Labor states that the *Mortierella* species might cause certain health effects such as: allergies, irritation, hypersensitivity pneumonitis, and dermatitis. Thus, the potential health risks associated with the presence of mould fungi inside buildings is a major concern for proprietors and, building administrators, as well as insurance companies.

Currently available anti-fungal agents dedicated to indoor applications must be non-toxic for humans and other animals, non-volatile, odourless, and hypoallergenic. Additionally, the chemistry of such agents should provide long-term protection, especially in environments that promote fungal growth (e.g. high humidity) (Clausen and Yang, 2007; Jarvis and Miller, 2005; Shoemaker and House, 2005). The presence of the fungal species leads to biological corrosion of the building materials. In order to inhibit or prevent the growth of microorganisms on these building materials, the use of relevant biocides is needed. However, many microorganisms become resistant to the hitherto applied chemicals. Therefore, there is a great need to develop new, more efficient antimicrobial agents for application in building material preservation. Silver nanoparticles (AgNPs) seemed to demonstrate strong antimicrobial properties. They started being widely used during the development of novel construction materials particularly, those materials that are excellent substrates for microorganism growth and development within specific environmental conditions. Recently, a few AgNPs containing products available on the market were used as antimicrobial agents to prevent bacterial growth, but little is known about their efficiency in respect to fungal growth (Chamakura et al., 2011; Choi et al., 2008; Kim et al., 2007 and Martínez-Castañón et al., 2008).

The objectives of the present study were to estimate the antifungal activity of silver nanoparticles in agar substrates and on gypsum wallboards. Morphological changes as well as the growth rate were described in the presence of increasing concentrations of AgNPs. Moreover, the diameters of conidiophores/sporangiophores were determined at the central part and edge of the mould colonies.

2. Materials and methods

2.1. Characterization of silver nanoparticles (AgNPs)

A solution of silver nanoparticles was obtained from NANOPAC Company (Krakow, Poland). Experimental concentrations of AgNPs were prepared from a stock solution (2000 mg/l) diluted with deionized water. Hydrodynamic sizes of AgNPs were determined at 25 °C using 173° dynamic light scattering approach (DLS, ZetaSizer Nano ZS). Visible/UV absorption spectra were recorded using a Hewlett–Packard HP 8452A diode-array spectrophotometer in 1 cm optical path cuvettes. The solution of Ag nanoparticles was analysed with the use of a Dimension Icon AFM (Bruker, Santa Barbara, CA) working in the PeakForce Tapping (PFT) mode with standard silicon cantilevers for measurements in the air (nominal spring constant of 0.4 N/m). Silver colloids were diluted 100 times and sonicated for 20 min prior to deposition. Silicon wafers were purified in "piranha" solution (a mixture of H_2SO_4 and H_2O_2 at a 1:3 ratio) and cleaned in water. The average size of nanoparticles reached 55 ± 5 nm. AgNP working solutions were added to the fungi culturing medium (PDA) or sprayed over gypsum drywall.

2.2. Sampling and identification of fungal species

Fungal species were isolated from affected basements of the Cracovian Market Square on Sabouroud medium containing Rose Bengal and purified by the sub-culturing on Potato Dextrose Agar (PDA, BD BBL™, Maryland, USA) or Czapek-Dox Broth (BD BBL™, Maryland, USA) prepared according to the manufacturer's protocol. Fungal species were identified on the basis of microscopy examinations and culture studies as previously described by Domsch et al. (1980). Identification was verified by sequencing their DNA at the ribosomal internal transcribed spacer (rDNA-ITS) region (Innis et al., 2012). DNA isolation was performed based on a modified method proposed by Kjøller and Rosendahl (2000). The nuclear ITS region of fungal DNA was amplified with the use of primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Then, pure DNA was bidirectionally sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (cat. 4337455, Applied Biosystems, USA). Reaction products were further purified with the use of EDTA–EtOH protocol. Finally, ITS sequences were analysed in 3130*xl* Genetic Analyser (ABi, USA). BLAST search (Altschul et al., 1997) was performed against sequences deposited in GenBank and the closest matches were selected for phylogenetic analysis.

2.3. Inoculum preparation

Suspension of spores obtained from *A. fumigatus*, *P. brevicompactum*, *C. cladosporoides*, *C. globosum* and *S. chartarum* or fragments of *M. alpina* mycelia were used as the inoculants. First, the fungal strains were cultured in Petri dishes on PDA substrate at 23 ± 1 °C for 14 days. Using a sterile inoculating loop, fungal spores were transferred from the stock culture to a 10 ml sterile physiological salt solution (0.85% NaCl, 0.03% Tween 80). The spore suspension was filtered through a 32 μm pore size nylon mesh (Sefar, Switzerland) to remove mycelia fragments, centrifuged at 13,523 *g* (Eppendorf centrifuge 5424, Germany), and supernatant was discarded. The spores were finally suspended in 50 ml of sterile physiological salt solution, but before inoculation, the number of spores was adjusted to 10^7 spores/ml by using a