The antifungal effect of silver nanoparticles on *Trichosporon asahii*

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**KEYWORDS**

Antifungal activity; Electron microscopy; Minimum inhibitory concentration; Silver nanoparticles; *Trichosporon asahii*

**Background/Purpose:** Silver nanoparticles are receiving increasing attention in biomedical applications. This study aims at evaluating the antifungal properties of silver nanoparticles against the pathogenic fungus *Trichosporon asahii*.

**Methods:** The growth of *T. asahii* on potato dextrose agar medium containing different concentrations of silver nanoparticles was examined and the antifungal effect was evaluated using minimum inhibitory concentration. Scanning and transmission electron microscopy were also used to investigate the antifungal effect of silver nanoparticles on *T. asahii*.

**Results:** Silver nanoparticles had a significant inhibitory effect on the growth of *T. asahii*. The minimum inhibitory concentration of silver nanoparticles against *T. asahii* was 0.5 µg/mL, which was lower than amphotericin B, 5-flucytosine, caspofungin, terbinafine, fluconazole, and itraconazole and higher than voriconazole. Silver nanoparticles obviously damaged the cell wall, cell membrane, mitochondria, chromatin, and ribosome.

**Conclusion:** Our results demonstrate that silver nanoparticles have good antifungal activity against *T. asahii*. Based on our electron microscopy observations, silver nanoparticles may inhibit the growth of *T. asahii* by permeating the fungal cell and damaging the cell wall and cellular components.

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Introduction

Nanotechnology is an emerging scientific field considered to have potential to generate new and innovative materials. Nanotechnology provides the ability to open new avenues to prevent and treat diseases by tailoring materials on an atomic scale. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment. Due to their unique properties, silver nanoparticles are receiving increasing attention in biomedical applications such as biological sensing, antimicrobial ointments, medical diagnostics, cancer therapy, drug delivery, and artificial implants.

Antimicrobial characteristics of silver ions and silver-based compounds have been reported for a variety of applications. Many studies have shown that silver nanoparticles are highly stable and toxic to bacteria, fungus, and viruses such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, *Candida albicans*, hepatitis B, human immunodeficiency virus-1, and syncytial virus. Carbon nanoscrolls AgNPs and graphene oxide AgNPs exhibit enhanced antifungal activities. Silver nanoparticles in combination with nystatin and chlorhexidine digluconate could enhance antifungal activity against *Candida albicans* and *Candida glabrata*. The release of silver ions from the crystalline core of silver nanoparticles contributes to the antimicrobial activity of these nanomaterials, making them useful for many applications in antimicrobial treatment.

*Trichosporon asahii* is an emerging fungal pathogen and has several growth forms: yeast-like round or oval spores, germ tubes, or pseudohyphae in response to environmental cues. In recent years, there have been even more reports about infection and drug resistance of *T. asahii*, which can invade the human body through implantable catheters, abraded skin, mucosa, or the respiratory tract, causing systemic and fatal trichosporosis. Trichosporon infections are associated with a wide spectrum of clinical manifestations, ranging from superficial cutaneous involvement in immunocompetent individuals to severe systemic diseases in immunocompromised patients. Due to limited availability of antifungal drugs, it is difficult to prevent and treat *T. asahii* infection. There is a strong incentive to develop new and more effective antifungal agents.

Although silver nanoparticles have shown great potential in biomedical applications, little is known about their antifungal effect on *T. asahii*, or their mechanism of action. Using a minimum inhibitory concentration (MIC) assay and electron microscopy (EM), we investigated the effect of silver nanoparticles on *T. asahii*. Our study provides a basis for using silver nanoparticles for potential prevention and treatment of *T. asahii* infections.

Materials and methods

Materials

A clinical strain (CBS2479) and several environmental strains (CBS58904, CBS7137, and CBS58520) of *T. asahii* were purchased from Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands). Further clinical strains (BZ701, BZ702, BZ703, BZ704, BZ705, BZ705R, BZ901, BZ902, BZ121, BZ122, BZ123, BZ124, and BZ125) were isolated from patients at General Hospital of Beijing Military Command (Beijing, China) that were infected by *T. asahii*. Potato dextrose agar (PDA) medium was used to grow and maintain the *T. asahii* cultures. A solution of silver nanoparticles (1000 ppm) was purchased from Huzheng Nano Technology Co., Ltd (Shanghai, China). Amphotericin B and terbinafine were purchased from Sigma–Aldrich (St Louis, MO, USA). Fluconazole and 5-flucytosine were purchased from Wako Chemicals (Richmond, VA, USA). Caspofungin was purchased from Merck Pharmaceutical Co., Ltd (Whitehouse Station, NJ, USA). Itraconazole was purchased from Xen–Janssen Pharmaceutical Ltd (Xi’an, China) and voriconazole was from Pfizer, Inc. (Hong Kong, China). Scanning EM (SEM) and transmission EM (TEM) images were obtained using a Hitachi H-3000 scanning electron microscope (Tokyo, Japan) and a Hitachi H-7650 transmission electron microscope (Tokyo, Japan), respectively.

Antifungal test

To examine the antifungal effect of silver nanoparticles, *T. asahii* cells were grown in PDA liquid medium at 35°C for 2 days, and then 1 x 10^6 colony-forming units/mL cells were plated on fresh PDA solid media containing different concentrations of silver nanoparticles (1 µg/mL, 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, 16 µg/mL, and 32 µg/mL) and incubated at 35°C for 5 days. Silver-free PDA plates, cultured under the same conditions, were used as controls. The sizes of the colonies were measured.

EM observation

The size and morphology of the bacteria were examined by SEM. Prior to SEM analysis, untreated and treated (4 µg/mL of silver nanoparticles) fungal cells were deposited on a Millipore filter and processed as previously described. In addition, TEM was used to examine the ultrastructure of the treated fungal cells, using standard procedures.

Measurement of MIC

The MIC of the antimicrobial activity of silver nanoparticles was evaluated according to the National Committee for Clinical Laboratory Standards. The antimicrobial effectiveness was determined against the final microorganism concentration of 10^6 colony-forming units/mL. Fungal growth was completely inhibited under the MIC. Amphotericin B (AMB), 5-flucytosine, caspofungin, terbinafine, fluconazole (FLC), itraconazole, and voriconazole were used as controls. All analyses were carried out in triplicate.

Results

Examination of the silver nanoparticles

The silver nanoparticles were examined using TEM (Fig. 1). Silver nanoparticles were round and had diameters of 5–20 nm.
Effects of silver nanoparticles on the growth of \textit{T. asahii}

Compared to the control group (no silver nanoparticles), the growth of \textit{T. asahii} was obviously inhibited by the silver nanoparticles in a concentration-dependent manner. The size of the colonies decreased with the increasing concentration of silver nanoparticles (Fig. 2). When the concentration of silver nanoparticles was $> 8 \, \mu g/mL$, no colony growth was observed on the plates. When the concentration was $< 2 \, \mu g/mL$, the silver nanoparticles showed no inhibitory effect on colony growth (data not shown).

**MICs of silver nanoparticles and other drugs against \textit{T. asahii}**

MICs of silver nanoparticles and other drugs against \textit{T. asahii} are shown in Table 1. The MIC of silver nanoparticles were estimated to be $0.5–1 \, \mu g/mL$, which were lower than those of AMB, 5-flucytosine, caspofungin, terbinafine, FLC, and itraconazole, but higher than voriconazole. There was no significant difference in MIC values of silver nanoparticles between clinical strains and environmental strains.

**Effects of silver nanoparticles on the surface morphology of \textit{T. asahii}**

SEM was used to evaluate the surface morphology of \textit{T. asahii}. In the absence of silver nanoparticles, hyphae of \textit{T. asahii} grew vigorously and had an average diameter of $3 \, \mu m$; the cell wall was intact (Fig. 3A). When treated with $4 \, \mu g/mL$ silver nanoparticles, the mycelium obviously deformed, distorted, shrunk, and fractured, and the surface of mycelium was damaged leading to the outflow of intracellular components and shrinkage of mycelium (Fig. 3B). The damage was more serious at higher concentrations of silver nanoparticles.

**Effects of silver nanoparticles on the ultrastructure of \textit{T. asahii}**

TEM was used to evaluate the ultrastructure of both the control and silver nanoparticle ($4 \, \mu g/mL$) treated \textit{T. asahii} cells. The cell structure of the untreated cell was intact (Fig. 4A). When treated with silver nanoparticles, cell wall and cell membrane structure was severely damaged (Fig. 4B–F). Dead cells were often observed, as suggested by the disappearance of the cell wall and cell membrane (Fig. 4G). Serious damage and degeneration of organelles was observed in silver-treated cells, including condensation and margination of chromatin, depolymerization of ribosomes, thinning of matrix, and fragmentation of mitochondria (Fig. 4H). Multivesicular bodies (Fig. 4I) and myeloid bodies (Fig. 4J), resulting from degeneration of organelles, were observed. The organelles were degraded, leading to empty space inside the cell (Fig. 4E). Silver nanoparticles were seen inside the cells (Fig. 4H and J) and on cell walls (Fig. 4K).

**Discussion**

The antimicrobial effects of silver have been known for many years. Over the past several years, the use of silver or silver salts as key components to control microbial proliferation has become increasingly popular. As a broad-spectrum antimicrobial agent, silver nanoparticles are widely used in medical and consumer products, including household antiseptic sprays and antimicrobial coatings for medical devices.

In this study, we demonstrated that the growth of the pathogenic fungus \textit{T. asahii} was inhibited by silver...
nanoparticles (Fig. 2). Similarly, a recent publication also showed that silver nanoparticles had antimicrobial effects against both Lactobacillus casei and T. asahii isolated from spoiled milk. Our electron microscopy analysis showed that silver nanoparticles damaged the cell wall and cell membrane, penetrated inside the cells, damaged the organelles including mitochondria and ribosome, and caused condensation and margination of chromatin, a marker of apoptotic cell death. Moreover, due to their small size, the silver nanoparticles may attach to the cell surface and get into the cells directly without damaging the cell wall and then cause the death of the cell. In a previous report on the bactericial activity of silver nanoparticles, it was shown that disruption of membranes of E. coli by silver nanoparticles significantly increases its permeability, leading to abnormal transport through the plasma membrane and, finally, cell death. Similar studies have also been reported in other fungal species, and mostly focused on the pathogenic fungus Candida albicans. To the best of our knowledge, this is the first report about inhibition of T. asahii by silver nanoparticles.

Several studies have reported that the electrostatic attraction between the negatively charged cell membrane of microorganisms, including bacteria, viruses, and fungi, and the positively charged nanoparticles is crucial for the antibacterial mode of these particles. It was believed that silver nanoparticles with large surface areas can easily form Ag+, binding to functional groups (-SH) of proteins and resulting in protein denaturation. Silver nanoparticles might also lead to protein denaturation and protein pump destruction by binding to the surface proteins of fungi, increasing the permeability of the membrane or protein lipid bilayer, finally resulting in disruption of the cell membrane. According to previous studies, it is likely that silver nanoparticles damage the transport system, causing efflux of intracellular ions and accumulation of

Table 1  Minimum inhibitory concentration (MIC) of silver nanoparticles and other common antifungal drugs against Trichosporon asahii

<table>
<thead>
<tr>
<th>Strains</th>
<th>NAG (μg/mL)</th>
<th>AMB (μg/mL)</th>
<th>5FC (μg/mL)</th>
<th>CAS (μg/mL)</th>
<th>TBF (μg/mL)</th>
<th>FLC (μg/mL)</th>
<th>ITC (μg/mL)</th>
<th>VRC (μg/mL)</th>
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<td>CBS2479</td>
<td>0.50 ± 0.00</td>
<td>5.33 ± 2.31</td>
<td>26.67 ± 9.24</td>
<td>32.00 ± 0.00</td>
<td>8.00 ± 0.00</td>
<td>1.67 ± 0.58</td>
<td>0.83 ± 0.29</td>
<td>0.06 ± 0.00</td>
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<td>BZ701</td>
<td>0.67 ± 0.29</td>
<td>13.33 ± 4.62</td>
<td>16.00 ± 0.00</td>
<td>21.33 ± 9.24</td>
<td>10.67 ± 4.62</td>
<td>1.67 ± 0.58</td>
<td>1.00 ± 0.00</td>
<td>0.05 ± 0.02</td>
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<td>BZ702</td>
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<td>13.33 ± 4.62</td>
<td>21.33 ± 9.24</td>
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<td>10.67 ± 4.62</td>
<td>1.00 ± 0.00</td>
<td>1.33 ± 0.58</td>
<td>0.04 ± 0.02</td>
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<tr>
<td>BZ703</td>
<td>1.00 ± 0.00</td>
<td>13.33 ± 4.62</td>
<td>16.00 ± 0.00</td>
<td>21.33 ± 9.24</td>
<td>10.67 ± 4.62</td>
<td>1.67 ± 0.58</td>
<td>0.83 ± 0.29</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>BZ704</td>
<td>0.67 ± 0.29</td>
<td>13.33 ± 4.62</td>
<td>21.33 ± 9.24</td>
<td>16.00 ± 0.00</td>
<td>10.67 ± 4.62</td>
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<td>1.67 ± 0.58</td>
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<td>BZ705</td>
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<td>8.00 ± 0.00</td>
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<td>1.00 ± 0.00</td>
<td>0.04 ± 0.02</td>
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<tr>
<td>BZ705 R</td>
<td>0.50 ± 0.00</td>
<td>1.67 ± 0.58</td>
<td>26.67 ± 9.24</td>
<td>16.00 ± 0.00</td>
<td>16.00 ± 0.00</td>
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<td>BZ901</td>
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<td>32.00 ± 0.00</td>
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<td>BZ902</td>
<td>1.00 ± 0.00</td>
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<td>21.33 ± 9.24</td>
<td>6.67 ± 2.31</td>
<td>1.67 ± 0.58</td>
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<td>BZ121</td>
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<td>42.67 ± 18.48</td>
<td>6.67 ± 2.31</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>0.06 ± 0.00</td>
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<tr>
<td>BZ124</td>
<td>0.67 ± 0.29</td>
<td>10.67 ± 4.62</td>
<td>26.67 ± 9.24</td>
<td>32.00 ± 0.00</td>
<td>5.33 ± 2.31</td>
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<td>0.67 ± 0.29</td>
<td>0.08 ± 0.03</td>
</tr>
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<td>BZ125</td>
<td>0.83 ± 0.29</td>
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<td>4.00 ± 0.00</td>
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<td>0.25 ± 0.00</td>
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<td>CBS7137</td>
<td>0.67 ± 0.29</td>
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<td>21.33 ± 9.24</td>
<td>10.67 ± 4.62</td>
<td>4.00 ± 0.00</td>
<td>1.67 ± 0.58</td>
<td>0.33 ± 0.14</td>
<td>0.04 ± 0.02</td>
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<tr>
<td>CBS8520</td>
<td>0.67 ± 0.29</td>
<td>32.00 ± 0.00</td>
<td>26.67 ± 9.24</td>
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<td>1.00 ± 0.00</td>
<td>0.42 ± 0.14</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.

NFC = 5-flucytosine; AMB = amphotericin B; CAS = caspofungin; FLC = fluconazole; ITC = itraconazole; NAG = silver nanoparticles; TBF = terbinafine; VRC = voriconazole.

Figure 3. Scanning electron microscopy micrographs of (A) untreated Trichosporon asahii (clinical strain CBS2479) and (B) T. asahii treated with a suspension of silver nanoparticles in potato dextrose medium (4 μg/mL) for 5 days.
silver ions, and blocking processes such as metabolism and respiration. Moreover, it is speculated that the bacterial cells in contact with silver nanoparticles may take up silver ions, which could facilitate the generation of reactive oxygen species and consequently cause cell death. Silver nanoparticles may also cause DNA damage and damage replication ability.2

In this study, we also found that an environmental strain was more sensitive to commonly used antifungal drugs (except AMB) than were clinical strains, possibly because the clinical strains have been exposed to certain antifungal drugs and therefore were resistant. This notion is further supported by the fact that the strain isolated from the patient treated with FLC and other drugs (BZ705R) was more resistant to drugs than the strain isolated from the same patient prior to drug treatment (BZ705). BZ705R has a higher MIC for FLC, further suggesting that exposure to certain medicine causes less drug susceptibility and

Figure 4. Transmission electron microscopy images showing the effects of silver nanoparticles on the ultrastructure of Trichosporon asahii. (A) The integral cellular structure can be seen in an untreated cell. (B–K) T. asahii was treated with 4 μg/mL of silver nanoparticles. (B) The cell wall was vague, (C) fell off, and (D) ruptured; (E) the cell membrane shrunk and the organelles were degraded, leading to the empty space inside the cell; and (F) rupture and dissolution. Red arrows (in B–D, and F) indicate the damage to cellular structure. (G) The cell wall and cell membrane disappeared, and only organelles could be seen within the cell. (H) Serious damage and degeneration of organelles was observed in silver-treated cells; (I) multivesicular bodies; and (J) myelin body. Silver nanoparticles can be seen both inside the cells (H and J, red arrows) and on cell walls (K, red arrow).
tolerance. However, the sensitivity of environmental and clinical strains to the silver nanoparticles was similar, and silver nanoparticles could inhibit the T. asahii growth more efficiently than the other six antifungal drugs, which are used widely in clinical practice. These results suggest that silver nanoparticles have good sterilization effect on both clinical strains and environmental strains. Thus, silver nanoparticles can be used as a potential antifungal agent for T. asahii. One concern is the possible cytotoxicity of silver nanoparticles to humans. 34 35 A number of studies have shown that silver nanoparticles have no cytotoxicity to human cells at low concentrations, but that increased doses might be cytotoxic. 35 37

In conclusion, we have demonstrated that silver nanoparticles exhibit excellent antifungal activity against T. asahii. However, the toxicity of silver nanoparticles to humans needs to be further evaluated in order to make them applicable to diverse medical devices and antimicrobial systems.

Conflicts of interest
All contributing authors declare no conflicts of interest.

Acknowledgments
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References


