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Antimicrobial textiles: Biogenic silver nanoparticles against *Candida* and *Xanthomonas*

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ABSTRACT

This paper introduces cotton fibers impregnated with biogenic silver nanoparticles (AgNPs), synthesized from a *Fusarium oxysporum* fungal filtrate (FF) solution, and open up the possibility for their use in medical environment and agriculture clothing as means to avoid microbial spreading. After thorough AgNPs characterization, regarding their physical, chemical and biochemical properties, Minimum Inhibitory Concentrations (MIC) against some human and orange tree pathogens were determined. We report the strong AgNPs activity against *Candida parapsilosis* and *Xanthomonas axonopodis* pv. *citri* (*Xac*) that was morphologically characterized, pointing to strong AgNPs effects on microorganisms' membranes. Cotton fibers were then impregnated with AgNPs suspension and these maintained strong antimicrobial activity even after repeated mechanical washing cycles (up to 10). Reported data might point to an application for biogenic AgNPs as potent agrochemicals, as well as, to their application in textiles for antiseptic clothing for medical and agronomic applications.

1. Introduction

Textiles can serve as a medium for bacteria and fungi growth and microbes transportation from one to another patient. Different strategies for antimicrobial finishing for textiles have been studied as a way to control microbe's growth and cross contamination [1]. Application of AgNPs in textiles for medical environments has gained attention worldwide, since these materials could be useful in the fight against multidrug resistant (MDR) bacteria and healthcare-associated infections (HAI) [2,3]. The ability of the impregnated textile on the reduction of microbial growth or elimination of microorganisms is useful in wound dressings, medical staff uniforms, bedsheet and others. Gerba et al. [4] describes the efficacy of silver impregnated textiles in the reduction of growth of different microorganisms. Ilic et al. [5] verified that cotton impregnated with 10–50 ppm of AgNPs had activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. However, they observed the release of AgNPs from the textiles in just few washing cycles. An alternative to the observed silver loss is the use of biosynthetic AgNPs capped with proteins, which allow a better AgNP adhesion to the textile's fiber. AgNPs produced by *F. oxysporum* were impregnated in cotton textile by Durán et al. [6] resulting in a reduction of 99.9% of colony forming units (CFU) of *S. aureus*, whereas Marcato et al. [7] verified a high antimicrobial activity even after 20 washing cycles.

AgNPs can be used in the reduction of infections in burns treatment, to avoid bacterial growth in medical equipment and textiles, and water treatment [8–12]. Biogenic AgNPs [16–21] are becoming important nanomaterials that have found their major use in antimicrobial applications [13–16]. Many efforts have been applied on the discovery of their mechanism of action [17]. It is most believed that the attachment of AgNPs to the surface of the cell membrane leads to cell death due to protein denaturation [18–20].

AgNPs stability may be attributed to the interactions between silver and proteins that leads to the formation of a biomolecular capping around the nanoparticle [21–33]. The biomolecular capping improves the interactions of the nanoparticles with microorganisms, increasing the antimicrobial activity [34]. Sintubin et al. [35] showed that biogenic AgNPs had 20 times more antimicrobial activity in comparison with chemical AgNPs.

Looking to the microorganisms, *Candida albicans* can cause infections in immunocompromised patients resulting in high mortality levels. The defence is based on *C. abicans* elimination by phagocytic cells [36] On the other hand, *Candida parapsilosis* is a human pathogen with increasing importance, since it leads to the invasive Candida disease. *C. parapsilosis* attacks mainly neonates and patients in intensive care units [37].

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Besides the biomedical applications, AgNPs have also been studied for agrochemical uses [38,39]. Citric Canker is a disease of significant economic importance worldwide, but no effective curative methods have been developed yet. Silver ions have already been studied for their use in formulations on the control of bacterial plant pathogens [40], however, to the best of our knowledge, this is the first study showing high in vitro activity of biogenic AgNPs against *Xanthomonas axonopodis* pv. *citri*, the causative agent of aforementioned disease.

Furthermore, nanotoxicology research is increasing substantially as nanoparticles are becoming widely used in different areas. The exposure to nanoparticles for biological and medical purposes involves direct contact, which makes the understanding of the nanoparticles' properties and their effects on the body crucial before their clinical use [41]. Since AgNPs are known to have antimicrobial activities, cytotoxicity assays are mandatory for a better understanding of their properties [42–43]. Moreover, in this article it is presented a complete study about biogenic AgNPs, including their biosynthesis and protein-capping characterization, antimicrobial activities using two different types of AgNPs against clinical pathogens and the causative agent of citric canker, nanosafety assays, including cytotoxicity tests and impregnated textiles for biomedical and agricultural usage.

2. Materials and methods

All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated. Fungal strain of Fusarium oxysporum (strain 551) was kindly provided from ESALQ-USP Genetic and Molecular Biology Laboratory (Piracicaba, Brazil).

2.1. Silver nanoparticles biosynthesis and characterization

The extracellular biosynthesis of AgNPs was performed following the method described by Durán et al. [44]. Differently, for the antimicrobial assays, two different types of AgNPs were prepared: $AgNP_1$, already described [44] and $AgNP_2$, obtained by washes with distilled water and triple centrifugation at 50,000 rpm for 30 min. The morphology of the biogenic silver nanoparticles was observed by Transmission Electron Microscopy (TEM). All images were taken in a Carl Zeiss CEM-902 (80 keV) microscope using 0.5 mg mL^{-1} AgNPs particle dispersion in water deposited on carbon-coated parlodion film supported in 300 mesh copper grids (Ted Pella). The average hydrodynamic diameter of nanoparticles was determined by dynamic light scattering (DLS) and the surface charge was measured in a Zetasizer Nano series equipment (Malvern Instruments). Zeta Potential was measured by electrophoretic mobility using the dispersions of the nanoparticles in a solution of KCl (USB) at 1.0 mmol L^{-1} concentration.

2.2. Fluorescence

Proteins from biogenic AgNPs and fungal filtrate (FF) were analysed in a Fluorescence Spectrophotometer Perkin Elmer (LS-55). Protein concentrations were 2 μmol L^{-1} for four samples, which were prepared in a phosphate saline buffer (PBS, 50 mmol L^{-1} and pH 7.4). The 10 mm path length cuvettes were used. With excitation at 280 nm, a band pass of 5 nm, the emission spectra were collected from 300 to 510 nm. The spectra baselines were corrected with the PBS buffer and data were an average of at least three independent experiments.

2.3. FTIR spectroscopy

Measurements were taken from KBr pellets of previously freeze dried samples of AgNPs and fungal filtrate, and recorded in an ABB Bomem (MB series) instrument with resolution of 4.0 cm^{-1}, in an interval from 4000 to 400 cm^{-1} and with 16 scans.

2.4. Raman spectroscopy

Measurements were recorded in a Horiba (T64000) instrument using two lasers in an average mode with five 1 scan for 900–3600 cm^{-1} and five 15 scans for a 200–900 cm^{-1} range. All analysed samples were previously freeze-dried.

2.5. Circular dichroism

Spectra, in near UV region (185–260 nm), were taken from 10 mm path-length square cuvettes in a JASCO (J-720) spectropolarimeter using samples with final protein concentration of 10 mmol L^{-1}.

2.6. Antimicrobial activity

The antimicrobial activity of AgNPs was evaluated against the fungi *Candida albicans* (ATCC 10231) and *Candida parapsilosis* (IFM48375), and bacterium *Xanthomonas axonopodis* pv. *citri* (IBSBF 1594) through determination of Minimum Inhibitory Concentration (MIC). For the determination of MIC values, 100 μL of AgNPs (stock solution of 0.1 mol L^{-1}) were sequentially diluted and to each microtube 50 μL (stock suspension of 0.5–2.5 × 10^3 CFU mL^{-1}) of the *Candida albicans*, *Candida parapsilosis* and *Xanthomonas axonopodis* pv. *citri* suspensions were added. Colonies of each pathogen were transferred to 15 mL test tubes containing 10 mL of 0.9% sterile saline solution and homogenized with vortex. The number of colony-forming units per mL (CFU mL^{-1}) present in solution was determined by absorption at 530 nm.

The pathogens were characterized by morphology (SEM) in the absence and presence of AgNPs using a JEOL (JSM-T300) Microscopy with 20 keV of acceleration and detectors for secondary and backscattered electrons. The sample was deposited in a sample port and recovered with gold by sputtering using a BALTEC sputter. The antimicrobial activity of the AgNPs was evaluated by liquid growth inhibition assay performed in microliter plates as described elsewhere [45,46].

2.7. Impregnated textiles

Cotton samples of 10 cm^2 were impregnated with the $AgNP_1$ suspension using the padding method. The fabrics impregnated twice were dried at room temperature for 24 h [6]. After impregnation, the fabrics were washed with 400 mg soap at 300 rpm for 15 min, and rinsed with 400 mL of distilled water at same conditions. At each wash process, water portions from the wash and rinse were stored in falcon tubes for ICP-AES. The analyses were conducted in a Perkin Elmer, Optima 3000 DV equipment to determine the silver leached. Moreover, portions of 1.0 cm^2 from the fabrics were taken at each wash cycle for EDS and SEM. The SEM analyses were done as described for the antimicrobial activity and the EDS was conducted in a Spectrometer EDX-700 from Shimadzu.

2.8. Antimicrobial activity of the impregnated textiles

The standard method for qualitative antimicrobial assessment of impregnated textiles is the AATCC 147. This method is based on the measure of diffusible antimicrobial agents on treated textiles. For this purpose, *Staphylococcus aureus* and *Klebsiella pneumoniae* are streaked onto agar plates and treated and untreated textiles are placed over the plates and incubated. The halo formed around the treated samples are measured in comparison with the untreated textile.

The antimicrobial activity of the impregnated textile fibers was studied qualitatively against *Candida albicans*, *Candida parapsilosis* and *Xanthomonas axonopodis* pv. *citri* through agar diffusion method as described before [47–49] and based on the AATCC 147. Each fabric portion of 1.0 cm^2 impregnated or not was placed in a Petri plate with Mueller-Hinton (*Candida*) and Luria-Bertani agar (*Xac*) at an appropriated temperature (37 °C) in the microorganism presence, previously inoculated