Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

INTERACTION OF *ASPERGILLUS NIGER* HYPHAE AND SPORES WITHCOLLOIDAL SILVER NANOPARTICLES

Ovidiu IORDACHE[1], Calina Petruta CORNEA[2]

[1]National Research and Development Institute for Textile and Leather, Lucretiu Patrascanu, No. 16, District 3, 030508, Bucharest, Romania
[2]University of Agronomical Sciences and Veterinary Medicine, Faculty of Biotechnologies, 59 Marasti Blvd., District 1, 011464, Bucharest, Romania

Corresponding author email: iordacheovidiu.g@gmail.com

Abstract

The experiments explored the interactions and antifungal properties of silver nanoparticles, against a model microorganism, Aspergillus niger. Scanning Electron Microscopy analysis were used for assessment of structural alterations done to fungal cells and hyphae, coupled with Energy Dispersive X-ray Spectroscopy (EDAX). The toxicity of the silver nanoparticles was tested using two methods: liquid exposure to the solution containing silver NPs, and spraying the NPs solution directly on the fungal culture. Analysis revealed significant cellular alteration due to the exposure to silver nanoparticles as well as effects on the growth of Aspergillus niger strain, in comparison to deionized water treatment, used at control sample. Microscopic SEM images revealed that silver nanoparticles treated hyphae were damaged on cell walls level, inducing plasmolysis, while EDAX analysis revealed strong silver depositions in the damaged areas of vegetative cells and spores walls, aspects that could be correlated with silver presence on the affected sites.

Keywords: *antimicrobial, silver, Aspergillus niger, SEM, EDAX.*

INTRODUCTION

Nanotechnology is currently employed as a powerful tool in aiding biomedical applications regarding antimicrobial activity. As known, the smaller a particle is, the greater it is surface area to volume ratio and the higher its biological activity and chemical reactivity. Metal particles in the nanometer size range exhibit physical properties that are different from both the ion and the bulk material. This makes them exhibit remarkable properties such as increased catalytic activity due to morphologies with highly active facets (Singh et al., 2008). The use of metal nanoparticles represents a quick and straightforward way of fighting against different types of microorganisms, colloidal silver being an effective bacteria-fighting agent (Gibbs, 1999).
The interaction between silver nanoparticles, and different microorganisms, is of outmost importance as a natural process that takes place in the nanometer scale region. Silver has long been known to exhibit a strong toxicity to a wide range of micro-organisms and for this reason silver-based compounds have been used extensively in many bactericidal applications (Singh et al., 2008).
Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. As an inhibitory mechanism, it has been proposed, that the silver easily adders to the cell wall, which at the exterior has nucleophile centers and alters cell respiration process. Ionic silver strongly interacts with thiol groups of vital enzymes which lead to their inactivation (Matsumura et al., 2003; Gupta et al., 1998). Through the transmembranar transport of the silver nanoparticles inside the cell, the functions of proteins and DNA are altered, which leads to the microorganism not being able to reproduce himself anymore. Studies underlined structural changes in the cell membrane and formation of small electron-dense granules formed by silver and sulfur (Feng et al., 2000; Nover et al., 1983). The positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles.

MATERIALS AND METHODS

Aspergillus niger (*A. niger*) was cultivated on a Czapek-Dox (2% w/v glucose, 2% w/v agar) media, at 29°C, for 14 days. The samples were kept on the agar media during the experimental procedure, in order to avoid mechanical damage that could influence the final results. After the culture was fully grown, the media containing the mature fungus was split in 2 equal halves, using a sterile spatula, one half for each method. The used colloid solution had a silver concentration of 1500 ppm, within a mix of styrenesulfonic acid and maleic anhydride. The silver nanoparticles were of colloidal shapes with an average size of 9 nm. For both methods, all samples were incubated 2 days at 29°C, with SEM and EDAX analysis being carried out after the incubation period. Before mounting, samples were washed carefully with deionized water. After stab mounting, the samples were allowed to dry in a desiccator at room temperature. For both methods, scanning electron microscopy (FEI Quanta 200) was used to observe changes in cell morphology after exposure to NPs. Also, EDAX (attached to a FEI Quanta 200 SEM) analysis was carried out to determine the deposition of silver nanoparticles on fungal cells. For liquid exposure method, the sample was fully sunken in the colloidal silver solution, thus being provided an equal coverage of the strain. For spraying method, the solution was sprayed over the media containing the mature fungus, using a pulveriser. In contrast to the previous method, this technique allows a much better O access. SEM images were taken after 2 days of exposure.

RESULTS AND DISCUSSIONS

In this study the antifungal activity of colloidal silver against *A. niger* cells was evaluated. *A. niger* was chosen due to its ubiquitous character and aggressive growing properties. The SEM images collected demonstrated that the silver nanoparticles inhibit cell wall integrity, as silver nanoparticles present a highly reactive potential. A study carried out by Morones et al. (2005) stated that silver nanoparticles disrupt transport systems, including ion efflux. The dysfunction of ion efflux can cause rapid accumulation of silver ions, interrupting cellular processes at their lower concentrations such as metabolism and respiration by reacting with molecules (Seon Min, et al., 2009) Sulfur-containing proteins from the cell wall are likely to be preferential sites for silver nanoparticles binding. Also, following silver nanoparticles transmembranar passage process, involved in a possible DNA binding process, the nanoparticles may have a role in gene inhibition, which may result in the cell not being able to reproduce anymore. Reports on the mechanism of inhibitory action of silver ions on microorganisms show that upon Ag treatment, DNA loses its replication ability and expression of ribosomal subunit proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated (Pal et al., 2009).The two different methods presented slightly different action sites, as fungal hyphae were more affected by the liquid exposure method, Figure 1, while fungal spores responded more efficiently to the spraying method, Figure 2.

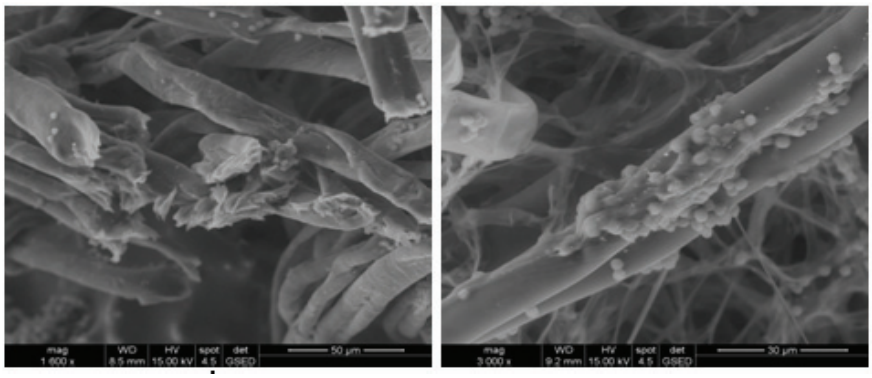

Figure 1. SEM images of sunken *A. niger* cells

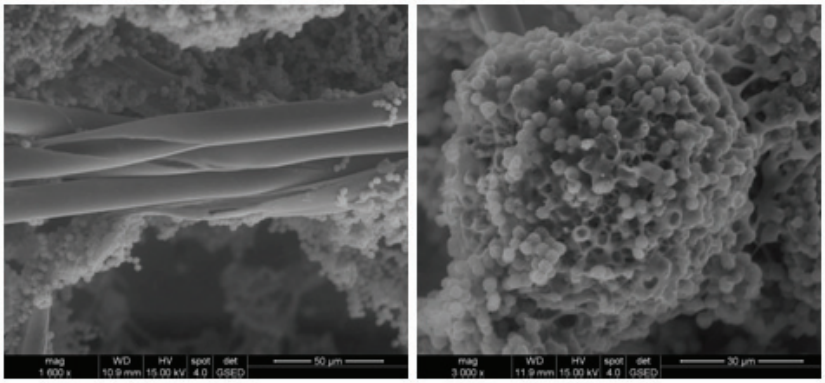

Figure 2. SEM images of sprayed *A. niger* cells

After silver treatment, microscopic images revealed that silver nanoparticles treated hyphae were damaged on hyphal walls, inducing hyphae plasmolysis, therefore cell lysis could be one of the reasons for the observed antibacterial property. For the witness samples treated with deionized water, no changes were noted varying the exposure period, Figure 3.

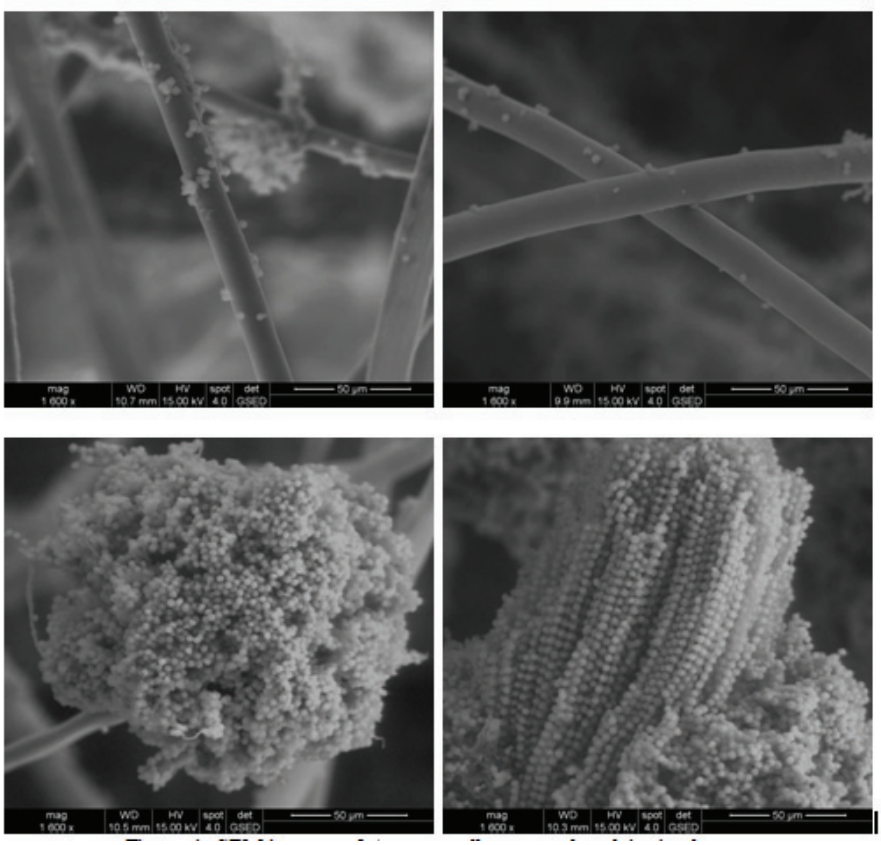

Figure 3. SEM images of *A. niger* cells exposed to deionized water

The Energy Dispersive X-Ray spectroscopy (EDAX) was used as an analytical technique used for elemental analysis and chemical characterization of the interaction between the culture and silver NPs. The analysis allowed identification of silver depositions in the damaged areas of hyphal and spores walls, Figure 4.

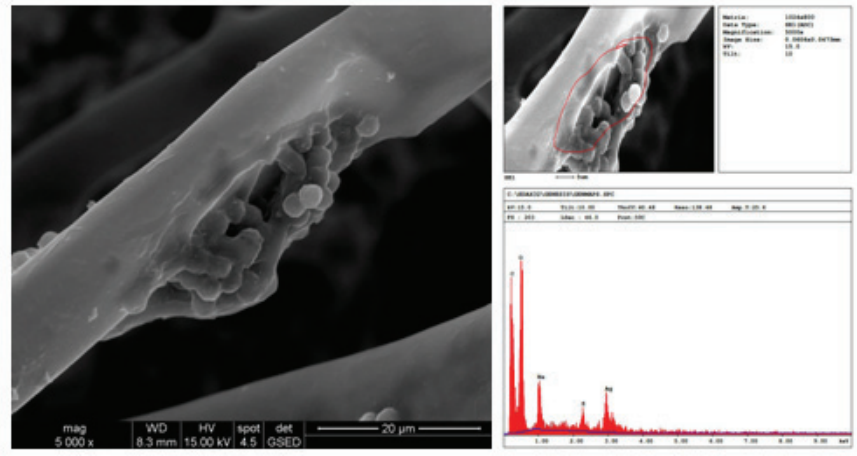

Figure 4. SEM and EDAX analysis of damaged hyphae and spores

CONCLUSIONS

The slightly different effect of the two methods may be explained be explained by a possible formation of silver nanoparticles aggregates, which in the case of the liquid exposure method they gather at the bottom of the Petri dish where they have a better access at the inferior part of the fungus culture. On the other hand, for the spraying method, before spraying the culture, the solution was well stirred, to provide a homogeneous distribution of silver nanoparticles within the solution. Therefore, after spraying, the conidial heads were covered in a solution "capsule", this way providing a better area of contact, in comparison with the fungus hyphae. The microscopic observations revealed that the silver nanoparticle solution clearly damaged fungal hyphae and spores, while the samples treated with deionized water appeared to remain intact.

The EDAX data acquisition was made from the damage site of fungal hyphae. The area or intensity of a peak in the acquisition spectrum is proportional to the concentration of the corresponding element in the sample. The peaks specific to carbon (C – 0.277 keV) and oxygen (O – 0.523 keV) have the largest share due to the organic character of the sample. The signal-peaks characteristic to sodium (Na-1.040 keV) and sulphur (S-2.307 keV) may be generated by proteins/enzymes present in the wall of the fungus, following cell lysis. Silver nanoparticles may have a crucial role in affecting the function of membrane-bound enzymes, those involved in the respiratory chain. This process can facilitate the generation of reactive oxygen species, which in the end can lead to cell death. The signal-peak found at 2.984 keV specific to silver (Ag) demonstrates the localization of silver in the damaged sites, thus strengthening the correlation between silver effect and hyphae and spores collapsing.

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